

COMPENDIUM

of OMAF Funded Food Safety Research

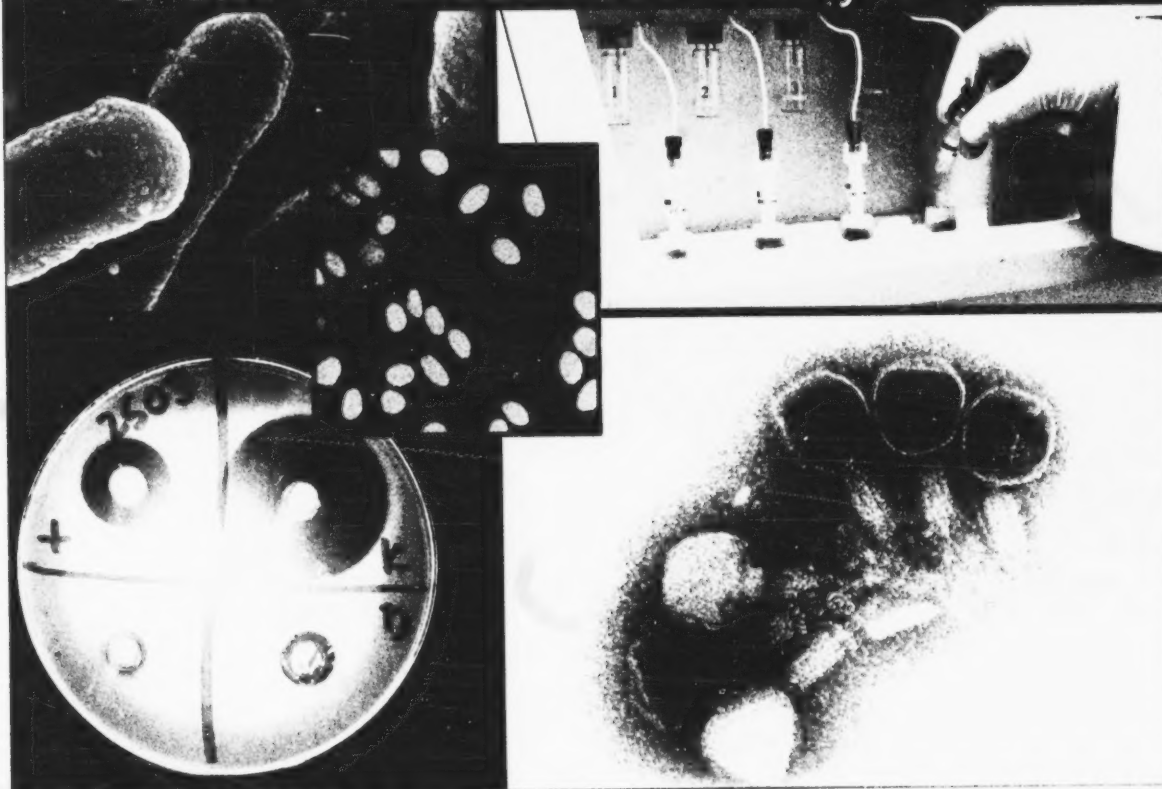


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Forward

Ontario has an excellent food safety track record. The Province of Ontario's Food Safety System initiative, developed in 1999, and the Food Safety and Quality Act, 2001, support the competitiveness of Ontario's agri-food industry and provide a framework to ensure Ontario has a science-based, food safety system which links the food chain from field to fork.

Research and development are important elements in maximizing the safety of Ontario's food. In 2002-03, the Ontario Ministry of Agriculture and Food (OMAF) will commit over \$3 million to food safety research and the commercialization of new technologies. This funding will support research to assess, manage and reduce the risks to public safety and livestock health along the entire food chain. It will identify emerging hazards and contaminants and develop/evaluate diagnostic methods to detect their presence in food and water. It will also generate knowledge that will help government regulators, industry, and consumers develop strategies to prevent, control or eliminate food safety risks.

The Ontario Ministry of Agriculture and Food supports food safety research and development through five initiatives:

- ☐ The OMAF-University of Guelph Agri-Food Research Program
- ☐ The Food Safety Research Program
- ☐ The Special Research New Directions in Agri-Food and Rural Research Program
- ☐ Healthy Futures for Ontario Agriculture
- ☐ Enhanced Food Quality and Safety Program

This *Compendium of OMAF Funded Food Safety Research* provides abstracts of 60 research projects which are in progress or have recently been completed at ten different research institutions and companies in Ontario. I encourage you to contact the project leader(s) listed in this compendium if you require further information about their research.

Finally, I would like to recognize and thank the many researchers, universities, federal government departments, provincial government ministries, organizations and companies that partner with OMAF to fund, perform and or communicate food safety research. This collaboration has helped to generate leading-edge research and new technologies that will benefit the Ontario agri-food industry, and the consumers of Ontario food, both at home and around the world.

Dr. Maurice Bitran
Director,
Innovation and Risk Management Branch
Ontario Ministry of Agriculture and Food

Section One: OMAF Programs Supporting Food Safety Research

OMAF - University of Guelph Agri-Food Research Program

The OMAF-University of Guelph Agri-Food Research Program is a \$33.5 million initiative that funds research in five program areas: Animal, Plant, Food, Sustainable Rural Communities and Resources Management and Environment. Four of these research program areas include projects that address food safety research priorities identified by stakeholders through the Ontario Agricultural Services Coordinating Committee and its sub-committees. The food safety research conducted through the OMAF - University of Guelph Agri-Food Research Program involves twelve different departments at the University, providing the broad range of expertise required to address food safety challenges along the entire food chain.

The total investment by OMAF in food safety research through the OMAF-University of Guelph Agri-Food Research Program in 2002- 2003 is \$1,635,274.

Further details on this program can be viewed at <http://www.uoguelph.ca/Research>

Table #1: Food Safety Related Projects Funded Through the Plant Research Program

Project Title	Project Leader
Production of antibodies in plants	Dr. Chris Hall, Environmental Biology, University of Guelph
Unregulated metals in biosolids: risks to the environment, plant and human health	Dr. Bev Hale, Land Resource Science, University of Guelph
Development of a food safety risk analysis model for issues management and risk communication	Dr. Doug Powell, Plant Agriculture, University of Guelph
Implementation and evaluation of on-farm food safety programs for the Ontario fruit and vegetable sector	
"Reduced risk" herbicides	Dr. John O'Sullivan, Plant Agriculture (Simcoe), University of Guelph

Table #2: Food Safety Related Projects Funded Through the Food Research Program

Project Title	Project Leader
Application of impedance technology for the detection and enumeration of <i>Salmonella</i> spp. on beef and poultry carcasses	Dr. Joseph Odumeru, Laboratory Services, University of Guelph
A systems approach to the evaluation and extension of ready-to-use packaged vegetables	Dr. Yukio Kakuda, Food Science, University of Guelph
Role of biofilm formation in the persistence of <i>Listeria monocytogenes</i> in food processing	Dr. Heidi Schraft, Dept. of Biology, Lakehead University

Characterization of foodborne pathogens using the RiboPrinter	Dr. Mansel Griffiths, Canadian Research Institute for Food Safety, Department of Food Science, University of Guelph
Improving the safety of fermented meat products	Dr. Shai Barbut, Food Science, University of Guelph
Fuzzy and statistical techniques for food safety risk characterization and valuation HACCP tools and integrated models for microbial risk assessment	Dr. Valerie Davidson, School of Engineering, University of Guelph
Process development for lower-fat and better-quality fried foods using edible coatings and for pan-fried meat patties for better safety	Dr. Gauri Mittal, School of Engineering, University of Guelph
Application of ozone for improving food quality and microbial safety	Dr. Hongde Zhou, School of Engineering, University of Guelph

Table #3: Food Safety Related Projects Funded Through the Animal Research Program

Project Title	Project Leader
<i>E. coli</i> 0157:H7 in veal calves in Ontario Characterization of <i>E. coli</i> 0157:H7 from pigs in Ontario	Dr. Carlton Gyles, Department of Pathobiology, University of Guelph
Prevention of microbial contamination in beef Enhancing the microbial food safety of pork	Dr. Scott McEwen, Department of Population Medicine, University of Guelph
Development of bovine specific probiotics for the prevention and treatment of <i>Escherichia coli</i> diarrhea in calves	Dr. Scott Weese, Department of Clinical Studies, University of Guelph
Epidemiology and impact of <i>Cryptosporidium</i> on Ontario dairy farms	Dr. Andrew Peregrine, Department of Pathobiology, University of Guelph
Investigation of the prevalence, diagnosis and management of Johne's disease in Ontario dairy herds	Dr. David Kelton, Department of Population Medicine, University of Guelph
Alternatives to raising pigs without antibiotics	Dr. Cate Dewey, Department of Population Medicine, University of Guelph
Enhancing the safety and quality of poultry products	Dr. Shai Barbut, Department of Food Science, University of Guelph
Avian mucosal lectins and resistance to <i>Salmonella</i> and <i>E.coli</i>	Dr. Tony Hayes, Department of Pathobiology, University of Guelph

Table #4: Food Safety Related Projects Funded Through the Resources Management and Environment Program

Project Title	Project Leader
Quantifying water quality impacts of livestock farms and other rural land uses	Ron Fleming, Ridgetown College, University of Guelph
Survival and release of fecal bacteria from rural stream sediments	Dr. Douglas Joy, University of Guelph
Implementation of food safety quality assurance schemes and an assessment of appropriate labelling for Ontario fruits and vegetables	Dr. Doug Powell, Plant Agriculture, University of Guelph
Construction of recombinant antibodies for detection of <i>Cryptosporidium parvum</i> .	Dr. H. Lee, University of Guelph

Food Safety Research Program

The Food Safety Research Program is a competitive, four-year, \$4 million research fund managed by the Innovation and Risk Management Branch of OMAF. It was launched in February 2001. The program is part of the Ontario Food Safety System initiative developed by three ministries: Ontario Ministry of Agriculture and Food (OMAF), Ministry of Natural Resources (MNR) and Ministry of Health and Long Term Care (MHLTC), in 1999. The initiative is designed to improve Ontario's food safety system by increasing the government's capacity to maintain high standards of food safety, protecting public health and increasing the marketability of Ontario food products.

The purpose of the program is to fund research to assist in the implementation of a science-based food safety system. The program priority areas of research eligible for funding are:

- Identification and analysis of emerging hazards and contaminants
- Development validation of testing methods
- Risk assessment, risk control and risk management

The research outcomes generated through this program will enhance knowledge and develop new technologies that will help scientists carry out systematic assessments of risk to public safety, support the implementation of HACCP and quality assurance programs and provide diagnostic tools to support field and laboratory testing for regulatory programs.

In the first two years (2000-2001 and 2001-2002), the program has allocated \$1.7 million to 13 research projects. Descriptions of funded research projects are available from the web site: http://www.gov.on.ca/OMAF/english/research/food/foodsafety_2001_index.html.

The process for the 2002-2003 round of proposals is almost completed and the program will allocate \$1.3 million to successful projects.

Priority areas of research are updated annually and described in the Research Requirements Document, which is published annually and posted on the web site: <http://www.gov.on.ca/OMAF/english/research>

Table 5 lists the projects funded through the Food Safety Research Program in Round I and Round II.

Table #5: Food Safety Related Projects Funded Through the Food Safety Research Program

Program Title	Project Leader
A community trial to determine an effective intervention for the delivery of HACCP to the food service sector	Dr. Tom Abernathy, Central West Health Planning Information Network
Johne's disease - new test validation	Dr. Marie Archambault, University of Guelph
Development of a rapid, sensitive and reliable test for the detection and quantification of <i>Escherichia coli</i> in foods	Dr. Stephen Brown, Queen's University
Development of a novel protein chip-based test for rapid and cost-effective <i>Salmonella</i> serotyping Improvement and validation of a DNA microchip-based test for rapid and simultaneous detection of six food-borne pathogens in food samples	Dr. Shu Chen, Laboratory Services Division, University of Guelph
Surveillance of Ontario pig farms for diseases of public health significance	Dr. Robert Friendship, University of Guelph
Assessing the incidence of antimicrobial resistant <i>E. coli</i> and <i>Salmonella</i> bacterial isolates in cull cows from Ontario free stall dairy herds	Dr. David Kelton, University of Guelph
Occurrence of enteric pathogens and antimicrobial resistance patterns in selected retail poultry products and human cases in a southwestern Ontario county <i>Campylobacter</i> isolation methodology and molecular characterization	Dr. Scott McEwen, University of Guelph
Development and validation of a screening protocol for identifying sulfamethazine-violative swine carcasses at Ontario abattoirs using the CHARM ROSA™ Sulfamethazine test	Mark Mitchell, University of Guelph
Application of the impedance and calorimetric systems for rapid and cost effective detection of <i>Listeria</i> spp. in food and environment samples	Dr. Joseph Odumeru, University of Guelph
Detection of <i>Campylobacter jejuni</i> by fluorescent <i>in situ</i> hybridization	Dr. Heidi Schraft, Lakehead University
Development of an analytical method for the confirmation of sulfonamides in animal tissues	Louise Spilsbury, University of Guelph

New Directions in Agri-Food and Rural Research Program (formerly the Special Research Program)

The New Directions in Agri-Food and Rural Research Program is an open, competitive research fund managed by the Innovation and Risk Management Branch of the Ontario Ministry of Agriculture and Food. Research projects are funded at a wide variety of public and private research institutions.

The purpose of the New Directions in Agri-Food and Rural Research Program is to stimulate the sustainable growth and competitiveness of Ontario's agri-food and rural sectors. This is accomplished through investment in innovative and high quality research in partnership with industry, rural communities organizations, other levels of government and research institutions. A wide variety of research priorities are addressed through this program, including issues related to food safety. Research priorities are established and published annually. Details on the New Directions Research Program can be viewed at: http://www.gov.on.ca/OMAF/english/research/new_directions_index.html

Table #6: Food Safety Related Projects Funded Through the New Directions/Special Research Program

Project Title	Project Leader
Modelling the survival of enterhemorrhagic <i>E. coli</i> in fermented meats and other meat products	Dr. Mansel Griffiths and Dr. Shai Barbut, University of Guelph
The development of novel DNA microchip techniques for use in diagnostics and food safety testing - Phase I The development of novel DNA microchip techniques for use in diagnostics and food safety testing - Phase II The development of DNA microarray technology for use in testing of key microbial contaminants in water samples	Dr. Shu Chen, University of Guelph
Development of a framework for antimicrobial use recording among Ontario beef producers Antimicrobial resistance in selected bacteria of beef cattle and associations with on-farm antimicrobial use	Dr. Scott McEwen, University of Guelph
Use of prebiotics and probiotics as replacements for antibiotics in pig starter feeds	Dr. Gregor Reid, Lawson Health Research Institute
Removal efficiency of representative bacterial and protozoan pathogens from cattle and pig farm wastewater using wetland technologies	Drs. Barry Warner and Robin Slawson, University of Waterloo
DNA fingerprinting analysis of <i>E. coli</i> using element and toxin specific gene amplification to differentiate pollution sources in the environment	Dr. Michele Van Dyke, GAP EnviroMicrobial Services
The effect of nutrient availability on biogeochemical processes affecting the transport of <i>Escherichia coli</i> in soils	Dr. Danielle Fortin, University of Ottawa
<i>Mycobacterium avium</i> Subspecies <i>Paratuberculosis</i> in the Ontario dairy industry: strategies for detection of the bacterium in dairy products	Drs. Lucy Mutharia and Joseph Odemeru, University of Guelph
Quantifying the preferential flow and recharge at the field scale: the first step in characterizing the nature and timing of pathogen transport to ground water	Dr. Gary Parkin, University of Guelph

Healthy Futures for Ontario Agriculture

The four-year, \$90 million Healthy Futures for Ontario Agriculture program, which closed to new applications on March 31, 2002, was created to support initiatives that will maintain and build on the success of Ontario's agri-food industry.

Developed by the Ontario Ministry of Agriculture and Food in co-operation with the province's agri-food industry, the program has focused on encouraging the agri-food industry to:

- enhance the safety and quality of Ontario food products
- capitalize on marketing and export opportunities
- improve rural water quality and make efficient use of rural water resources

At the time of publishing this compendium, two major research and development projects with a total OMAF investment of \$2,565,536 have been announced (see Table 7). Further details on the Healthy Futures for Ontario Agriculture initiative are available at: <http://www.gov.on.ca/OMAF/english/hfoa/index.html>

Table #7: Food Safety Related Projects Funded Through the Healthy Futures for Ontario Agriculture Program

Project Title	Project Leader
Vaccination of cattle to reduce fecal shedding, environmental and water contamination with <i>E. coli</i> O157:H7	Martin Warmelink, Bioniche Life Sciences Inc.
FOXA biosensor for water quality	Dr. Paul Piuino, Virtek Vision International Inc.

Enhanced Food Quality and Safety Program

The Enhanced Food Quality and Safety Program (EFQSP) was administered by OMAF between 1994 - 2000. Its goal was to improve the efficiency and effectiveness of the Laboratory Services Division (LSD), University of Guelph in providing greater service to the agri-food industry in the areas of environmental sustainability, food safety and food quality, and animal and plant disease diagnostics. Specific research areas were targeted to ensure that the analytical service and development efforts of the LSD are responsive to the needs of the Ontario agri-food industry. The intent of the research priorities was to develop or evaluate specific techniques and methodologies that could be readily incorporated into the laboratory facility and could be used to improve and in some cases, expand services of a world class analytical laboratory centre. Research was targeted in the following areas:

- Food Chemistry
- Food Microbiology
- Risk Assessment/Epidemiology/Pre-harvest Food Safety
- Pesticides and Trace Contaminant Analysis

Table 8 lists the projects funded through the Enhanced Food Quality and Safety program that are still in progress or have been recently completed. For a complete listing of research supported through this program (prior to 1999-2000) visit the web page at <http://www.gov.on.ca/OMAF/english/research>.

Table #8: Food Safety Related Projects Funded Through the Enhanced Food Quality and Safety Program, 1999-2001

Project Title	Project Leaders
Development of an analytical method for the quantitative detection of cephalosporin residues in bovine milk	Louise Spilsbury, University of Guelph
Development of a surface water testing procedure to determine species of origin for <i>Escherichia coli</i> bacteria	Dr. Joseph Odumeru, University of Guelph
Evaluation of impedance microbiological method the detection of <i>Campylobacter</i> species in commercial broiler parent flock and broiler environments	Dr. Joseph Odumeru, University of Guelph
Development of a method for detection of <i>Cyclospora cayentensis</i> in raspberries and other foods	Dr. Joseph Odumeru, University of Guelph
Effect of pesticides in recycled water used in mushroom compost preparation on mushroom production and quality	Dr. Greg Duns and Dr. Danny Rinker, University of Guelph

Section Two: Abstracts

Diagnostic Methods - Development and Validation (DM)

DM01 - Project Title: Johne's disease - new test validation

Project Leader: Dr. Marie Archambault, Animal Health Laboratory (LSD), University of Guelph, Guelph ON N1H 6R8
Tel: 519-824-4120 x 4536 E-mail: marchamb@lsd.uoguelph.ca

Collaborating Researchers: Dr. Grant Maxie, Dr. Hugh Cai, Dr. David Kelton, Dr. Davor Ojkic, Dr. Jocelyn Jansen

Project Duration: June, 2001 - May, 2003

Abstract:

Paratuberculosis (Johne's disease) is an infectious disease of ruminants caused by *Mycobacterium avium* spp *paratuberculosis* (*M. paratuberculosis*) considered by many to be the most serious infectious disease currently plaguing the world's cattle, sheep, and goat industries. In the terminal stages of the disease, affected cows develop diarrhea and wasting. More importantly, most infected cows are asymptomatic but some will shed the organism in colostrum and milk as well as contaminate the environment by fecal shedding. In addition to animal health, a significant concern to consumers and to Ontario dairy producers is the controversial evidence that *M. paratuberculosis* may be causally linked with Crohn's disease of humans. Control and elimination of *M. paratuberculosis* from Ontario dairy herds will greatly reduce the possibility of milk transfer and hence consumer concerns, and will assist with domestic and international sales of dairy and beef cattle, especially to prominent US dairy states with active Johne's control programs.

Control of Johne's disease has been complicated by the absence of rapid, cost-effective and sensitive individual or herd tests. The Johne's ELISA is rapid, but about 15% of infected animals may not be antibody positive, even in late-stage infections. The BACTEC culture system offers speed benefits over conventional culture, which requires at least 16 weeks to complete because of the extremely slow growth of *M. paratuberculosis*. Recently, IDEXX Laboratories launched an improved Johne's DNA probe for PCR.

The overall objective of the Johne's project is to provide rapid and accurate diagnostic tests to identify cattle infected with *M. paratuberculosis* in Ontario. The first goal is to set up and validate the BACTEC system and the IDEXX PCR DNA test kit to identify *M. paratuberculosis* in bovine feces. The last objective is to combine both procedures to reduce the turnaround time of diagnostic tests for Johne's disease.

After the analytical methods were developed for both the BACTEC culture and the IDEXX PCR, the testing of fecal samples began. Fieldwork was done in collaboration with the Department of Population Medicine at the Ontario Veterinary College. Thirty-one herds (2101 cows) were bled and an ELISA test was conducted on all of samples. Two hundred and sixty-nine cows (13.1%) were positive. A total of 241 fecal samples were provided to our laboratory as well as to the AntelBio laboratory for traditional culture; 240 more samples are to be added. Currently, 241 samples have been processed and are being tested weekly on the BACTEC TB460; positives have been detected. As well, the 241 fecal samples are being processed directly by PCR with the IDEXX DNA test kit. At the end of 2003, an evaluation of BACTEC, traditional culture, fecal PCR and serum ELISA diagnostic tests for Johne's disease will be completed. It is expected that BACTEC culture and PCR diagnostic tests will then be available to veterinary practitioners in Ontario at the Animal Health Laboratory.

DM02 - Project Title: Development of a rapid, sensitive and reliable test for the detection and quantification of *Escherichia coli* in foods

Project Leader: Dr. Stephen Brown, School of Environmental Studies Centre for Water and Environment, Queen's University, Kingston ON K7L 3N6 Tel: 613-533-2655 E-mail: browns@chem.queensu.ca

Collaborating Researcher: Dr. Andrew Kropinski, Queen's University

Project Duration: September, 2001 - August, 2003

Abstract:

Escherichia coli in fecal matter has been known to contaminate food and to cause poisonings. However, current tests typically require a 24-hour incubation period and can take 2-3 days before results are communicated. The goal of this project is to develop a rapid and sensitive, yet simple and reliable test for the identification and quantification of *E. coli* in foods. Almost one year has been completed on this research project and a summary of the results achieved to date are described below.

The test is a modified version standard *E. coli* test where a sample is incubated for up to 24 hours in a solution containing a fluorogenic substrate, such as 4-methylumbelliferyl- β -glucuronide (MUG). If *E. coli* bacteria are in the sample, they will grow and produce the enzyme β -glucuronidase, which then hydrolyzes the substrate to produce a fluorescent product (in this case, 4-methylumbelliferone). Presence or absence of the fluorescent product (and hence presence or absence of the bacteria) is determined by observing the sample under an ultraviolet lamp.

The new fibre-optic test system uses a custom designed substrate compound and a fibre-optic probe designed to detect the unique product generated when *E. coli* consumes the substrate. Fluorescence is continually monitored by the computer-controlled instrument, instead of at a fixed time after incubation, providing the earliest possible indication of a positive sample. Human judgment of the fluorescence intensity is not required, and the probe can extract the optical signal even from samples which are coloured, turbid or have other fluorescent substances in them. In addition, by tracking the fluorescence signal over time, an estimate of the number of cells present in the initial sample can be made, providing a quantitative test. Highly contaminated samples can be confirmed positive in as little as 1-2 hours.

In the first phase of this project, several candidate substrates and growth media for bacterial detection using laboratory strain *E. coli* 413 were tested. In the second phase, water samples will be analyzed using the research team's system and a membrane filter method at a local accredited laboratory. Each lab takes a 10 ml aliquot from the same sample (after shaking). Thus far, 37 samples have been tested and the results summarized on a presence absence basis. The summary is 12 negative samples, 19 positive samples, and 6 samples showing negative in one lab but only 1 cell in the other lab (statistically in agreement), indicating excellent performance of the test on a presence absence basis. Comparison of the quantitative results is also promising, though more data is needed to be definitive.

The next phase of this project includes work on food samples, beginning with juice, vegetable wash and meat wash samples which have been spiked with *E. coli* cells. Meat swipes and meat homogenates will be added to test those more complex matrices. Finally, food samples will be tested in parallel with a local accredited laboratory to verify the test performance on real samples.

DM03 - Project Title: The development of novel DNA microchip technologies for use in diagnostics and food safety testing - Phase I

Project Leaders: Drs. Shu Chen and David Evans, Laboratory Services Division and Department of Molecular Biology and Genetics, University of Guelph, Guelph ON N1G 8J7
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Collaborating Researchers: Dr. Susy Carmen, Laboratory Services Division, University of Guelph; and Ms. Beverly Grammara, Virtek Vision, Waterloo, ON

Project Duration: August, 1999 - August, 2000

Abstract:

Rapid, simple and cost-effective detection of human pathogens in food and clinical samples is crucial in ensuring food safety and public health. Recent developments in nucleic acid-based technologies have led to the availability of the DNA microchip (or microarray) technology that allows researchers to develop cost-effective and revolutionary types of molecular analyses for the detection of multiple pathogens in a single test. The DNA microchip technology employs arrays of DNA probes to simultaneously analyze massive numbers of target nucleic acid sequences by hybridization.

The purpose of this research was to acquire and develop the DNA microchip technology for use in diagnostics and food safety testing. In this project, a complete DNA chip system (Brown Lab Standford arrayer and Virtek Vision's ChipReader™) was installed at the University of Guelph. The associated methods for microarray fabrication, target amplification, DNA hybridization and fluorescent detection were established. The probes suitable for arraying were produced by PCR RT-PCR, and immobilized onto aldehyde-activated microscopic slides to form microarrays. The Cy3- or Cy5-labeled fluorescent DNA targets were prepared by multiplex PCR RT-PCR assays and hybridized to the probe DNAs, and the arrays were scanned to determine the positive target binding site(s). Two model chips were developed for the detection of model pathogens, Shiga toxin-producing *Escherichia coli* (STEC) and Influenza virus. The functionality of the model chips was demonstrated.

The first model chip was developed for simultaneous detection and identification of multiple types of Shiga toxin-producing *E. coli* and *E. coli* serotype O157:H7. The microarray consisted of twenty specific DNA probes that contained the sequences of the toxin genes *stx1*, *stx2* and *stx3* variants, the *uidA* gene, the 16S rRNA gene and a serotype O157:H7 specific DNA marker. An associated fluorescent multiplex PCR procedure was developed to simultaneously amplify all the DNA targets involved in the hybridization.

The microarray multiplex PCR system was demonstrated to be able to detect and differentiate the Shiga toxin types and the serotype O157:H7 using seven reference strains. The system was evaluated using 52 additional *E. coli* isolates. The hybridization images of the 52 isolates showed 7 different patterns that correctly identified their toxin types and the serotype O157:H7.

The second model DNA microarray was developed to facilitate typing and subtyping of human influenza A and B viruses. The microarray contained twenty-six probes encoding the hemagglutinin, neuraminidase, and matrix protein genes of influenza virus. The microarrays were tested with 12 cloned cDNA preparations. The hybridization patterns agreed perfectly with the identities of the clones. Combinations of Cy3- and Cy5-labeled target DNAs were also prepared and hybridized to the same chip, permitting further differentiation of amplified molecules in complex mixtures. In a more realistic test of the technology, three sets of multiplex PCR primers that collectively target influenza A and B virus strains were identified and were used to type and subtype three previously unsequenced influenza virus isolates.

It was concluded from this research that the DNA microarray technology is a powerful tool for rapid and potentially cost-effective diagnostics of multiple pathogens in a single test.

DM04 - Project Title: The development of novel DNA microchip technologies for use in diagnostics and food safety testing - Phase II

Project Leaders: Drs. Shu Chen and David Evans, Laboratory Services Division and Department of Molecular Biology and Genetics, University of Guelph, Guelph ON N1G 8J7 Tel: 519-767-6319 Email: schen@lsd.uoguelph.ca

Collaborating Researchers: Dr. Dongwan Yoo, Department of Pathobiology, University of Guelph; Ms. Kris Rahn, Health Canada; Dr. Susy Carmen, Laboratory Services Division, University of Guelph; and Dr. Reda Fayek, Virtek Vision, Waterloo, ON

Project Duration: August, 2000 - August, 2001

Abstract:

In the previous project (or Phase I of the project), a DNA chip system was installed at the University of Guelph and developed two model chips for the detection of model pathogens (Shiga toxin-producing *E. coli* and influenza virus). In this project, the chip technology was moved one step forward from the initial "proof of concept" phase to development of practical DNA chips. The bacterial model chip was expanded to cover additional pathogens including *Campylobacter*, *Salmonella*, *Salmonella typhimurium* DT104 and *Listeria monocytogenes*, and a virus chip was developed to monitor gene expression of poxviruses for the purpose of identifying better diagnostic targets of the viruses.

The bacterial microarray consisted of twenty-nine synthetic oligonucleotide probes that contained the DNA sequences of the Shiga toxin genes of *E. coli*, the *tadA* gene and an *E. coli* serotype O157:H7 specific marker, the *invA* gene of *Salmonella*, the *Stx1* gene of *S. typhimurium* DT104, the *fliA* gene of *Campylobacter*, the *hlyA* gene of *L. monocytogenes* and the bacterial 16S rRNA gene. An associated fluorescent multiplex PCR assay was designed to simultaneously amplify all of the target genes in a single PCR reaction. The PCR microarray system was demonstrated to be able to correctly detect and differentiate the major Stx types and the serotype O157:H7 of *E. coli*, and to detect *Campylobacter*, *Salmonella*, *S. typhimurium* DT104 and *L. monocytogenes* for ten reference and twenty non-reference strains tested. The system was also tested using nine naturally contaminated meat (beef) samples for the presence of Shiga toxin-producing *E. coli*. The microarray results agreed with the results obtained using the standard culture method. It was concluded that the multiplex PCR-microarray system is capable of detecting the six pathogens in a single test, and should potentially be a valuable tool for cost-effective food safety testing. However, further studies are required to further optimize all the procedures involved in the assay before the system can be validated extensively and then implemented in food testing laboratories.

The focus of the virus microarray development was to explore whether DNA chip methods can be used to identify DNA viruses through an analysis of viral gene expression patterns. Poxvirus was used as a target because of the extraordinary demand for new poxvirus diagnostics that has been generated by recent concerns over the possible use of variola (smallpox) as an agent of biological terrorism. The virus microarray consisted of DNA probes that contained sequences of all of the open-reading frames encoded by vaccinia virus (except D5R). The probes were prepared using the PCR method as developed in our previous project. The chip was successfully hybridized to cDNA prepared using RNA extracted from vaccinia virus-infected cells at 12 hr post-infection. Late transcripts were easily detected by this method. The most important conclusion from this work is that the transcripts from genes like A12L, A14L, A17L, A26L, and A27L would be superior targets for rtPCR-based poxvirus detection schemes. The results also provide a basis for further research into whether virus transcriptome patterns can serve as the basis for novel diagnostics.

DM05 - Project Title: The development of DNA microarray technology for use in testing of key microbial contaminants in water samples

Project Leader: Dr. Shu Chen, Laboratory Services Division, University of Guelph, Guelph, ON N1H 8J7
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Collaborating Researchers: Dr. Mansel Griffiths, Department of Food Science, University of Guelph; Ms. Kris Rahn, Laboratory for Foodborne Zoonoses, Health Canada; Dr. Brian Brooks, Canadian Food Inspection Agency; Dr. Reda Fayek, Virtek Vision, Waterloo, ON

Project Duration: October, 2001 - January, 2003

Abstract:

The key contaminants in surface and ground water resources, including *Cryptosporidium*, *Giardia*, *Cyclospora*, *Escherichia coli*, *Salmonella*, *Listeria*, and *Campylobacter*, are common causes of waterborne diseases. Rapid, simple and cost effective detection of these key pathogenic contaminants is crucial in ensuring water safety and a healthy environment. The purpose of this research is to develop a microchip-based test for detection of these contaminants in water samples.

This project is an extension of the previous two projects. In these projects, a "prototype" DNA chip was developed for simultaneous detection of the bacterial pathogens including Shiga toxin producing *E. coli* (STEC), *E. coli* serotype O157:H7, *Campylobacter*, *Salmonella*, *S. typhimurium* DT104, and *Listeria monocytogenes*. In this project, the prototype chip will be expanded to cover the intestinal parasites including *Cryptosporidium*, *Giardia* and *Cyclospora*.

Twenty-five specific DNA probes and six primers targeting the parasites have been developed and prepared based on the oocyst wall protein-encoding (CpR1) gene for *Cryptosporidium*, the β -giardin gene for *Giardia* and the internal transcribed spacer (ITS) region of the rRNA operon of *Cyclospora*. A multiplex PCR assay has been formulated to simultaneously amplify all the parasitic target genes in one reaction. Fluorescent PCR products have been successfully hybridized to the appropriate spots on the microarray using three control strains of the three parasites. The multiplex PCR microarray system has been tested using additional six *C. parvum* strains and one *Eimeria* strain. The hybridization images have allowed correct detection of the *C. parvum* strains, and showed no cross-hybridization with the *Eimeria* strain.

The probes have been combined with the bacterial probes that have been prepared in another on-going project to produce an array for simultaneous detection of nine water- and food-borne pathogens. The expanded microbial arrays were hybridized with the fluorescent target DNA samples prepared from 18 reference strains of Shiga toxin-producing *E. coli*, *E. coli* serotype O157:H7, *Salmonella*, *S. typhimurium* DT104, *L. monocytogenes* and *Campylobacter*, as well as the parasites *C. parvum*, *G. intestinalis* and *C. cayentensis*. The results have showed that all of the target strains could be detected and distinguished. No cross-hybridization was observed with any of the strains tested. The performance of the multiplex PCR microarray system is being evaluated for its specificity and sensitivity using more microbial strains as well as spiked water samples. It is expected that successful completion of the project will result in a DNA microchip-based test that can potentially be used for simultaneous detection of the key water and food-borne pathogens in a single test.

DM06 - Project Title: Improvement and validation of a DNA microchip-based test for rapid and simultaneous detection of six food-borne pathogens in food samples

Project Leader: Dr. Shu Chen, Laboratory Services Division, University of Guelph, Guelph, ON N1H 8J7
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Collaborating Researchers: Dr. Joseph Odumeru, Laboratory Services Division, University of Guelph; Dr. Roger Johnson, Laboratory for Foodborne Zoonoses, Health Canada; Dr. David Chiu, Department of Computing and Information Science, University of Guelph; Dr. David Evans, Department of Molecular Biology and Genetics, University of Guelph and Dr. Reda Fayek, Virtek Vision, Waterloo, ON

Project Duration: August, 2001 - January, 2003

Abstract:

Current diagnostic assays only allow for detection of a single pathogen at a time in food samples. However, there are a number of food-borne pathogens that are today's major public concerns. The DNA chip or microarray-based technology allows for simultaneous detection of large numbers of pathogens in a single sample. In previous projects, a prototype microarray-based test was developed for simultaneous detection of six common food-borne bacterial pathogens in a single test, including Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* serotype O157:H7, *Salmonella*, *Salmonella typhimurium* DT104, *Listeria*

monocytogenes and *Campylobacter*. The purpose of this project is to improve, optimize and validate the prototype microchip and associated procedures so that the chip-based test can eventually be used in diagnostic settings.

The major progress that has been made so far includes: (1) optimization of DNA probes on the chip that has improved probe specificity and hybridization efficiency, and allowed differentiation between *Campylobacter coli* and *jejuni*; (2) modification of the Stanford Brown arrayer and optimization of spotting conditions that have resulted in much higher spot quality and reproducible results; (3) improvement of the DNA extraction and PCR product purification methods, and optimization of hybridization conditions that have allowed completion of the test within 24-48 hours (upon receiving food samples); (4) optimization of the multiplex PCR assay conditions that has balanced amplification efficiency among the DNA targets and reduced the limit of detection of the assay to less than 2×10^3 colony forming units.

The system has been partially validated for its specificity using 188 organisms, including 45 isolates of *E. coli* serotype O157:H7, 36 non-O157:H7 STEC, 17 *E. coli*, 47 *L. monocytogenes*, 5 *S. typhimurium* DT 104, 30 non-DT104 *Salmonella* strains, and 8 other non-target microorganisms. Hybridization images demonstrated 187 matches, and only one *E. coli* strain was classified as *C. jejuni*. The sensitivity of the microarray hybridization was tested to be approximately 10 to 25 times higher than regular agarose gel electrophoresis. The system has been validated using more target and non-target organisms, as well as a large number of naturally contaminated food samples. A database is being established based on the validation results and customized software will be created for effective data management and interpretation.

The final outcome of the project will be a chip-based test and its associated software that can be used for rapid, specific and simultaneous detection of six food-borne pathogens in a single test in food samples. The availability of such tests will allow diagnostic labs to provide faster and cost-effective results to agri-food industries and consumers and provide an avenue to enhance the safety of Ontario's food supplies. This will also allow testing laboratories and government inspection staff to conduct risk assessment studies in a cost-effective and systematic manner in support of monitoring and surveillance activities and enforcement of regulatory programs. In addition, this will help to provide faster results for diagnosticians trying to identify the culprits behind foodborne diseases.

DM07 - Project Title: Development of a novel protein chip-based test for rapid and cost-effective *Salmonella* serotyping

Project Leader: Dr. Shu Chen, Laboratory Services Division, University of Guelph, Guelph, ON N1H 8J7
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Collaborating Researchers: Dr. Hugh Cai, Laboratory Services Division, University of Guelph; Dr. Anne Muckle, Laboratory for Foodborne Zoonoses, Health Canada; Phil Nafekh, Virtek Vision, Waterloo, ON

Project Duration: June, 2002 - May, 2004

Abstract:

Salmonella is one of the most important agents of food-borne illnesses. It consists of over 2400 serotypes. Serotyping is the most important universal typing method for characterization of *Salmonella* isolates, and involves more than 100-250 antisera for identification of all serotypes. The current *Salmonella* serotyping method only allows for detection of a single antibody-antigen reaction at a time. A minimum of six antibody-antigen reactions are needed for recognition of a particular *Salmonella* serovar. The number of reactions required can be several times greater if a less common serotype is under testing. The assay consumes high volumes of reagents, takes a minimum of three days to provide an answer and can take as long as two to three weeks to settle some multiphase serotypes. The microchip or microarray-based technology allows for simultaneous detection of large numbers of molecule interactions in a single test with minimal reagent consumption. The technology would be an ideal technology platform for *Salmonella* serotyping.

A DNA microarray-based test for simultaneous detection of multiple food-borne pathogens in a single test has been successfully developed. In this project, a protein microarray-based test for rapid, simple, accurate and cost-effective serotyping of *Salmonella* will be developed. In Phase I of the project, the techniques for protein-based array construction and detection will be developed. The Phase II of the project will involve development and validation of two model arrays for detection of O and H antigens respectively using representative *Salmonella* antisera and strains and establishment of procedures and criteria for making and using an array(s) for serotyping. In Phase III of the project, an array system for serotyping of commonly identified and clinically important *Salmonella* serovars will be developed and optimized. The specificity and sensitivity of the system using a limited number of *Salmonella* and non-*Salmonella* bacteria will be determined. The effectiveness of the typing system will be verified with the standard Kauffmann-White typing scheme.

Successful completion of the project will result in a rapid, simple, accurate and cost-effective protein chip based-test that can be used for serotyping of commonly-identified and clinically important *Salmonella* serovars upon further validation. The availability

of such a test will eventually allow diagnostic labs, such as Laboratory Services Division, University of Guelph, to provide faster and cost-effective results to food and animal industries, and to improve the efficiency and effectiveness in providing greater services to Ontario's agri-food industries in the areas of food safety and animal disease control. This will also allow testing laboratories and government agencies to conduct baseline and outbreak studies in a cost-effective and timely manner. This will eventually provide an avenue to enhance the safety of Ontario's food supplies, and help to ensure that the Ontario agri-food industries remain competitive in providing safe foods in meeting the needs of today's food safety conscious consumers.

DM08 - Project Title: Characterization of foodborne pathogens using the RiboPrinter

Project Leader: Dr. M. Griffiths, Canadian Research Institute for Food Safety, Department of Food Science, University of Guelph, Guelph ON N1G 2W1 Tel: 519-824-4120 x 2269 Email: mgriffit@uoguelph.ca

Project Duration: May, 2000 - April, 2003

Abstract:

The RiboPrinter is an automated instrument for genotyping bacteria and large databases of riboprint patterns of several foodborne pathogens are being accumulated which will be invaluable for disease surveillance. These riboprints can be generated within 4 to 8 hours once an isolate has been obtained. It is being used to characterize and type bacteria during outbreak investigations and to determine the source of bacterial contamination in food processing plants. For example, the RiboPrinter was instrumental in identifying contaminated hot dogs from Sara Lee's Bil Mar, Michigan plant as the source of a large listeriosis outbreak in the US in 1998. It has also been suggested that the RiboPrinter can be used to rapidly serotype *Salmonella* based on their ribotype.

At the American Society for Microbiology meeting held in Los Angeles (2000), results were presented which showed that the RiboPrinter could be used to identify human pathogenic strains of Shiga-like toxin producing *E. coli* (SLTEC). In view of events at Walkerton, a rapid method to identify these dangerous pathogens is urgently needed. A method to rapidly source contamination by this pathogen would also be very useful and a method is being sought to differentiate EHEC based on their animal reservoir. This study will investigate the use of the RiboPrinter to differentiate SLTEC and other foodborne pathogens. The project specific objectives include the following:

1. to investigate the use of the RiboPrinter to differentiate human pathogenic strains of Shiga-like toxin producing *E. coli* (SLTEC)
2. to investigate the use of the RiboPrinter to source animal reservoirs of *E. coli*
3. to investigate the use of the RiboPrinter to serotype *Salmonella*.

A method has been developed to type *S. enteritidis* and *E. coli* 0157:H7 to allow better identification of outbreak strains and monitoring in production and processing environments. The development of rapid typing methods for *Salmonella*, *E. coli* 0157:H7 and *Listeria monocytogenes* will allow detection of sources of contamination of foods at all points along the food chain in a timely manner. This will improve food safety and minimize economic losses to industry due to recalls and litigation.

DM09 - Project Title: Production of antibodies in plants

Project Leader: Dr Chris Hall, Department of Environmental Biology, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May 2000-April 2006.

Abstract:

In response to recent concerns about food safety, the public is demanding chemical- and pathogen-free food, a clean environment, and healthy alternatives to conventional medicine and cheaper, more effective diagnostic and therapeutic products. Antibodies, due to their high degree of specificity and binding capacity for a diverse range of targets, offer significant potential in applications, including food safety, medical diagnostics, therapeutics, plant biotechnology and the detection of environmental contaminants. Traditionally antibodies have been derived from animal sources, however, low yields, high cost and concerns over the scientific use of animals make these methods impractical and socially unacceptable. This project is looking to replace animal-derived antibodies with those produced in plants. The first step will be to create a basic research platform for screening and maturing antibodies and then produce and purify antibodies on a large scale from genetically modified tobacco. The basic research platform will provide high quality antibodies for applications in environmental detection and monitoring, food safety, discovery of new lead chemistries, purification of nutraceuticals and pharmaceuticals and animal and human immunotherapy. The aim is to develop innovative agricultural, biotechnological and diagnostic products for protection of the environment and improving human health and well-being. This will involve making cost effective products using plant-derived antibodies against specific food-borne pathogens. These antibodies will be used to signal to a customer if the food has become contaminated and hence is unsafe to eat. Plant-expressed antibodies could also be used to protect the public from biological terrorism for which immunization is not practical.

The project specific objectives are as follows:

1. production of antibodies in *E. coli*-based bacterial expression systems against major food borne pathogens i.e. *Listeria monocytogenes*, *E. coli* strain 0157:H7, *Salmonella* and the waterborne parasite, *Cryptosporidium parvum*
2. movement of these genes from bacteria for low-cost production of large scale amounts of antibodies in plants against the same food borne and waterborne pathogens
3. incorporation of plant derived antibodies on plastic material used in the food industry
4. demonstration of the utility of these novel packaging systems in detecting food borne pathogens
5. evaluation of field grown tobacco for plant antibody yield
6. Small and large scale purification of antibodies from transgenic tobacco.

DM10 - Project Title: Construction of recombinant antibodies for detection of *Cryptosporidium parvum*

Project Leader(s): Dr. H. Lee, Department of Environmental Biology, University of Guelph, Guelph, ON N1G 2W1
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Collaborating Researchers: Dr. J. L. Trevors and Dr. C. Hall, University of Guelph

Project Duration: May, 2000 - April, 2003

Abstract:

The enteric protozoan *Cryptosporidium* is an important and widely distributed pathogen causing the gastrointestinal illness cryptosporidiosis in both humans and several species of farm animals. Water is a common vehicle for transmission of *Cryptosporidium* spp. In the past few years, several recent waterborne outbreaks of cryptosporidiosis have occurred in Ontario due to run-offs from agricultural fields or human fecal contamination. These outbreaks were of significant concern to health authorities and highlighted deficiencies in their ability to respond to the public's concern about the safety of Ontario's water supplies. Although different species of the parasite exist, *Cryptosporidium parvum* appears to be the most widely distributed and commonly implicated in clinical disease outbreaks. Reliable detection of *Cryptosporidium parvum* is essential for routine monitoring and prevention of cryptosporidiosis. This collaborative project will construct recombinant antibodies against *C. parvum*. The general methods to be used for construction of scFv or Fab recombinant antibodies will be based on those already established in previous research and described in Webb et al. (1997) and Yau et al. (1998). The recombinant antibodies produced in this study will then be tested and compared with other preparations of commercial mabs in the immunofluorescence assay-based specific detection of *C. parvum* in laboratory and environmental water samples.

If successful, a ready source of cheap bacterial recombinant antibodies will be available that can be used for the specific, sensitive and effective detection of *C. parvum* in environmental samples.

DM11 - Project Title: *Campylobacter* isolation methodology and molecular characterization

Lead Researcher: Dr. Scott McEwen, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Collaborating Research Establishment(s): Laboratory for Foodborne Zoonoses, Health Canada; Division of Enteric, Foodborne and Waterborne Diseases, Bureau of Infectious Diseases, Health Canada; Clinical and Environmental Microbiology Department, Central Public Health Laboratory, Health Services Division, Ontario Ministry of Health and Long-term Care

Project Duration: September, 2001 - August, 2003

Abstract:

There are two main objectives of this project. The first objective is to further investigate the relationship between the *Campylobacter* spp. isolated from the human and chicken samples utilizing molecular characterization techniques. A subset of human and poultry isolates with similar antimicrobial resistance profiles will be evaluated using PCR-DNA sequence analysis of a short variable region within the *fla* gene. This will provide more complete and definitive information for the analysis of the relationship between antimicrobial resistance in retail poultry and human isolates and the risk factors associated with human campylobacteriosis.

The second objective is to investigate and compare the performance of the current method for enumeration of *Campylobacter* spp. from carcass rinse samples with the Campy-Cefex Agar method from both carcass rinse samples and package weep fluid samples. The Campy-Cefex Agar method is a faster and more economical method than is currently utilized in research situations.

The additional advantage of the package weep fluid sample is the ease of sample collection and the non-destructive nature of the sampling. Enumeration of *Campylobacter* spp. in retail poultry provides information on the level of risk to which the consumer is exposed. This information is an important component of risk analysis and risk assessments. Currently, enumeration is not practical for inclusion in surveillance or the majority of research projects due to the extensive resource and time commitments of the official method. The availability of a validated, rapid and inexpensive enumeration method would provide the opportunity for enumeration to be included in surveillance and research initiatives.

The results contribute to the maintenance of Ontario's standards of food safety and preservation of public health by: providing a rapid and inexpensive method for enumeration of *Campylobacter* in meat; improve our understanding of the potential transmission of antimicrobial resistant strains of *Campylobacter* via the foodchain; and provide the basis for further initiatives including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant strains of *Campylobacter* in retail poultry.

DM12 - Project Title: Development and validation of a screening protocol for identifying sulfamethazine-violative swine carcasses at Ontario abattoirs using the CHARM ROSA™ Sulfamethazine test

Project Leader: Mark Mitchell, Laboratory Services Division, University of Guelph, Guelph ON N1H 8J7
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Collaborating Researcher(s): Louse Spilbury, Laboratory Services Division, University of Guelph

Project Duration: January, 2002-January, 2003

Abstract:

Sulfonamides are widely used in the swine industry for disease treatment and growth promotion. The use of sulfamethazine (SMZ) in combination with sub therapeutic levels of antimicrobials is particularly common in starter (weanling pig) rations. As a result, the risk of SMZ residues in young animals such as barbecue hogs that may not have been allocated a long enough withdrawal period before slaughter is high.

The Ontario Ministry of Agriculture and Food (OMAF) has extensive drug residue surveillance and monitoring programs for animals slaughtered at provincial abattoirs. These programs involve taking tissue samples directly from suspect and randomly selected carcasses for analysis. In the case of market and barbecue hogs, a urine screen for SMZ (SOS or 'Sulfa On Site' test) is used in order to more accurately target carcasses requiring confirmatory testing.

Because pork is a relatively perishable product, the screening program for SMZ residues in hogs attempts to minimize turnaround time by shipping the urine of slaughtered animals to the laboratory for testing via 24-hour courier service. The vast majority of carcasses (ie. 97.9% during January to April 2001) currently pass the SOS test. This means that a large number of marketable carcasses are needlessly held in storage at the abattoirs using valuable storage space and increasing the time to move this perishable product to market. A rapid and simple on-site SMZ screening test allowing for the identification of non-violative carcasses is therefore highly desirable.

The SOS test is a thin layer chromatographic method, developed by the United States Department of Agriculture (U.S.D.A.) in 1986, and is considered an effective tool for identifying carcasses containing violative levels of SMZ. However, it possesses certain drawbacks that make it undesirable for use in an abattoir environment. These include availability problems and its dependence on a toxic, flammable solvent (ie. ethyl acetate) that is particularly hazardous in surroundings lacking the specialized ventilation safeguards of a laboratory.

A suitable urine screening test for abattoir use should be rapid, easy and safe to use, and able to identify carcasses containing potentially violative levels of SMZ (ie. > 100 ppb Maximum Residue Limit (MRL) for porcine tissue and organs). The CHARM ROSA™ (Rapid On Site Assay) is an immunochromatographic assay intended for the detection of SMZ residues in raw milk. Preliminary work at the University of Guelph lab indicates that this test may be adaptable for use in swine urine. To meet specific performance requirements, the manufacturers have optimized the test for its intended purpose. Any changes in the recommended testing procedure, such as a change in the test matrix, can significantly alter the performance of the assay. Therefore, it is essential that a test be thoroughly evaluated before it is incorporated into a regulatory testing program (i.e. determination of test sensitivity and selectivity, ruggedness, cross reactivity and comparison to an accepted method).

In this study, the CHARM ROSA™ SMZ test will be adapted and validated for the screening of urine samples in Ontario abattoirs, thereby, allowing the separation of carcasses (edible muscle) containing violative vs. non-violative levels of SMZ. Suspect tissue samples can then be forwarded to the lab for confirmatory testing.

DM13 - Project Title: *Mycobacterium avium* subspecies *Paratuberculosis* in the Ontario dairy industry: strategies for detection of the bacterium in dairy products

Project Leader(s): Dr. L. Mutharia and Dr. J. Odumeru, Department of Microbiology and Laboratory Services Division, University of Guelph, Guelph ON N1G 2W1 lmuthari@uoguelph.ca and jodumeru@lsd.uoguelph.ca

Collaborating Research Establishments Industry Partners: Dairy Farmers of Ontario

Project Duration: October, 2001 - October, 2003

Abstract:

The goals of this study are to provide a diagnostic assay and generate useful information for farmers that can be applied to: 1) detecting contamination of dairy products with the bacterium *Mycobacterium avium* subspecies *paratuberculosis* or MAP; and 2) the long term management of MAP transmission within herds. To meet these goals the researchers plan to develop an assay that combines bacterial-capture technology, with the "gold-standard" of culture and rapid detection of MAP, nucleic acids. The advantages of this technology are, differentiating contamination of food with live from dead MAP, expedited detection of live bacteria using the BACTEC technology, and expedited detection of the bacterium using molecular technologies.

The objectives of the study are to:

1. Optimize sample decontamination protocols for capture and culture of MAP cells. Currently used protocols reduce the viability of this fastidious bacterium by up to 50%.
2. Develop protocols for direct extraction of MAP DNA from samples.
3. Define the minimum incubation periods for detection of viable MAP.
4. Generate baseline information on the prevalence of MAP in dairy products, to assist the Dairy Farmers of Ontario in the management of MAP transmission in herds.

In the long term, this project is of benefit to the management of Johne's disease, to farm production and to increase consumer confidence in the dairy industry. The assay could be applied to identifying MAP in the environment and provide a useful tool for the maintenance of a healthy environment on dairy farms.

DM14 - Project Title: Development of a surface water testing procedure to determine species of origin for *Escherichia coli* bacteria

Project Leaders: Dr. Joseph Odumeru, Laboratory Services Division, University of Guelph, 95 Stone Road West, Guelph, ON N1H 8J7 Tel: 519-767-6243 Email: jodumeru@lsd.uoguelph.ca and Dr. Carlton Gyles, Department of Pathobiology, University of Guelph

Collaborating Researchers: Dr. S. Chen, University of Guelph, Dr. S. Martin, University of Guelph, Dr. R. Xu, University of Guelph

Project Duration: May, 1999 - April, 2001

Abstract:

Counts of coliform bacteria in water indicate the potential presence of pathogenic microbes of intestinal origin but give no indication of the origin of the microbial pollution. The objective of this research was to develop a method for differentiating *E. coli* isolates of livestock, wildlife, or human origin that might be used to predict the sources of fecal pollution of water. A collection of 380 *E. coli* isolates from the feces of cattle, poultry, swine, deer, goose, and moose as well as from human sewage or clinical, samples was used to evaluate four methods. One method was the multiple-antibiotic-resistance (MAR) profile, using 13 antibiotics. Discriminant analysis revealed that 81% of the livestock isolates, 68% of the wildlife isolates and 61% of the human isolates were assigned to the correct source groups. Amplified fragment length polymorphism (AFLP), the second test, was applied to 105 of the *E. coli* isolates. The AFLP results showed that 94% of the livestock isolates, 100% of the wildlife isolates, and 91% of the human isolates were correctly classified. The third method was repetitive palindromic sequence based polymerase chain reaction (rep-PCR) in which repetitive sequences of 199 the *E. coli* genome were amplified. Analysis of rep-PCR correctly assigned 66% of animal, 69% of poultry, and 70% of human isolates. Sequence analysis of the 16S rRNA genes of the *E. coli* isolates was the fourth method evaluated in this work. Discriminant analysis of 105 *E. coli* isolates indicated that 78% of livestock isolates, 74% of wildlife isolates, and 80% of human isolates could be correctly classified into their host groups. Overall, these results indicate that AFLP was the most effective of the four methods that were evaluated.

DM15 - Project Title: Evaluation of impedance microbiological method for detection of *Campylobacter* species in commercial broiler parent flock

Project Leader: Dr. Joseph Odumeru, Laboratory Services Division, University of Guelph, 95 Stone Road West, Guelph, ON N1H 8J7 Tel: 519-767-6243 Email: jodumeru@lsd.uoguelph.ca

Project Duration: September, 2000 - September, 2001

Abstract:

Current culture methods for the detection of *Campylobacter* are labour demanding and time consuming, requiring 6 - 7 days for confirmation of positive results. In this study, a more rapid and less labour intensive method, the Impedance Microbiological Method, was evaluated for the detection of *Campylobacter* in poultry products and facilities. The Malthus Microbiological Analyzer system (Malthus System V, Malthus Instruments Ltd., Bury, United Kingdom) was compared to the Hunt microbiological culture procedure. When the Malthus *Campylobacter* Selective Growth Medium was spiked with pure cultures of *Campylobacter* species, the detection limit of the Malthus Analyzer was as low as 1 CFU/cell but *E. coli* and *S. typhimurium* were detected at 10⁷ CFU/cell. The sensitivity and the specificity of the Impedance Microbiological Method as well as the Impedance Method combined with culture confirmation were evaluated for *Campylobacter* detection in naturally contaminated poultry carcass rinses and environmental samples. The sensitivity of the Impedance Method was 100% for both the carcass rinses and the environmental samples but the specificity was only 21.6% for carcass rinses and 36.4% for environmental samples. When the Impedance Method was combined with subsequent culture confirmation, the sensitivity was 97.3% for the carcass rinses and 100% for the environmental samples, and the specificity was 100% for both sample types. These results indicate that the Impedance Microbiological Method for detection of *Campylobacter* in poultry samples must be combined with culture confirmation procedures to obtain reliable results.

DM16 - Project Title: Development of a method for detection of *Cyclospora cayentanensis* in raspberries and other foods

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Collaborating Researcher: Dr. Heidi Shraft, Lakehead University

Project Duration: September, 2000 - September, 2002

Abstract:

Cyclospora cayentanensis is a newly emerging pathogen, which has recently received considerable attention as a cause of large outbreaks of persistent diarrhea within Canada and the United States. The ecology of *C. cayentanensis* is not completely understood, although the parasite is believed to be endemic in several countries, including Guatemala, and Peru. *Cyclospora cayentanensis* infection in North America is strongly associated with consumption of produce from these countries, particularly with raspberries from Guatemala. To prevent future outbreaks it is necessary to develop improved detection methods which could then be used in trace-back investigations where *C. cayentanensis* is endemic. A polymerase chain reaction (PCR) detection assay for *C. cayentanensis* has been developed which is fairly sensitive and specific. Further modification to this method to improve the sensitivity would increase its usefulness as a detection method for produce. This method could then be used to conduct a survey to determine the incidence of *C. cayentanensis* in produce from Canada and from endemic countries to evaluate the risk of infection for consumers.

DM17 - Project Title: Application of the impedance and calorimetric systems for rapid and cost effective detection of *Listeria* species in food and environmental samples

Project Leader: Dr. Joseph Odumeru, Laboratory Services Division, University of Guelph, Guelph ON N1H 8J7 Tel: 519-767-6243 Email: jodumeru@lsd.uoguelph.ca

Project Duration: May 2001 - April 2003

Abstract:

Listeria species are ubiquitous in nature, especially *L. monocytogenes* and *L. innocua*. Several outbreaks of food borne listeriosis due to *L. monocytogenes* have been reported, with severe economic and health impacts. *Listeria* species are common in the food industry environment including food handlers. A variety of food products including dairy, meat and poultry, vegetables and seafood have been shown to be contaminated with *Listeria* species and the plant environment is implicated in the majority of cases. Although in-coming raw materials are the primary sources of *Listeria* spp., these organisms persist in a wet, humid plant

environment with faulty sanitation. *Listeria monocytogenes*, the pathogenic species of *Listeria*, has been shown to form biofilms in various food-processing surfaces. Biofilm growth of *L. monocytogenes* provides a substantial risk of pathogen contamination especially if wet surfaces are not maintained in a sanitary condition. Many food and meat processors test environmental swabs and sponges to confirm the absence of *Listeria* species, as part of their GMP and HACCP verification programs. Culture method, the most commonly used method, requires up to seven days to complete and does not fit well into these programs which require immediate remedial action. This study will be assessing the application of two semi-automated systems, impedance-based system (Malthus) and a calorimetric- based system (Biosys) for rapid and low-cost detection of *Listeria* species, in food and environmental samples. The swab and sponge samples collected from a food processing plant which monitors for *Listeria* spp., specifically *L. monocytogenes* and *L. innocua*, the two most prevalent listerias in food processing environments will be tested. Also, vegetable and poultry samples submitted for routine testing at the Laboratory Services Division, Food Microbiology lab, University of Guelph, will be tested for these organisms. To date 350 environmental swabs have been tested. Preliminary results showed an excellent correlation between the rapid detection systems and culture method. Successful validation of the application of these systems for detecting *Listeria* spp. in food and environmental samples will help determine which of these is the most cost and time efficient system, with acceptable sensitivity and specificity for *Listeria* testing. Recommendations will be made regarding the use of these systems for rapid and cost-effective screening of environmental samples and food products for *Listeria* spp.

DM18 - Project Title: Application of impedance technology for the detection and enumeration of *Salmonella* spp. on beef and poultry carcasses

Project Leader: Dr. Joseph Odumeru, Laboratory Services Division, University of Guelph, Guelph ON N1H 8J7
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Project Duration: May, 2000 - September, 2001

Abstract:

Previous studies in the University of Guelph Lab have demonstrated that the impedance microbiological method can be used as an alternative method to the conventional culture method for the detection of bacteria in foods. A recent study from the same Lab was able to detect *Escherichia coli* at low levels (< 1 CFU/gm) in a variety of food products including raw and ready-to-eat meats using the impedance microbiological method. The method is based on the measurement of changes in weak electrical current between electrodes held in broth culture in specialized cells, to detect the growth of bacteria in the medium.

In this study the application of this technology for the rapid detection of *Salmonella* spp. in beef and poultry samples within 48 hours of testing was determined. Results showed that the impedance microbiological method using the newly developed LS *Salmonella* medium had a detection limit of approximately $< 1 - 10$ *Salmonella* organisms per ml of culture medium. Results of tests of spiked ground beef samples indicate that as low as 1 CFU of *Salmonella* organisms per gram of beef samples can be detected. The impedance system using LS medium detected all seven different serovars of *Salmonella* tested ($< 1 - 10$ *Salmonella* organisms/ml or gm of sample). Tests for *Salmonella* organisms in naturally contaminated beef carcass and poultry environmental samples showed 100% sensitivity and specificity for beef carcass samples and 88 - 100% sensitivity and 100% specificity for poultry environmental samples using a combination of impedance measurements, media color change due to growth of *Salmonella*, and culture confirmation of presumptive positive results. While the sensitivity of the impedance measurements for poultry carcass rinses was 100% (no false negative), only 60% of impedance positive samples were confirmed culture positive by conventional culture method. *Salmonella* organisms were not consistently recovered from the poultry carcass rinses tested by the system and the level of false positive was unacceptably high. Therefore, the system is not recommended for use in this type of food matrix. Further modification to increase the selectivity of the media is required in order to obtain acceptable specificity and sensitivity for poultry carcass rinses.

In conclusion, the Malthus impedance method with LS medium is a useful method for screening for the presence of *Salmonella* in beef carcass and poultry environmental samples. Only "suspect" samples need to be confirmed by subsequent culture confirmation procedures. This impedance method reduced the time required to complete test from 6 days to 48 hours and the labor required to detect and isolate *Salmonella* from beef and poultry environmental samples compared to conventional culture techniques.

DM19 - Project Title: FONA sensor for water quality

Project Leader: Dr. Paul Piuino, FONA Technologies Inc. (A wholly owned subsidiary of Virtek Laser Systems Inc.), 2395 Speakman Drive, Mississauga, ON, L5K 1B3, Tel: 905.822.5286, Email: paul.piuino@virtek.ca

Collaborators: GAP EnviroMicrobial Services (a division of Conestoga Rovers and Associates)

Project Duration: February, 2002 - December, 2003

Abstract:

This project aims to develop and commercialize a fibre-optic nucleic acid (FONA) biosensor for testing water quality that is rapid, quantitative, flexible and which can be deployed in testing labs, treatment facilities and ultimately in the field. The FONA biosensor is currently in second prototype development phase.

The FONA sensor for water quality project starts from a second generation FONA instrument capable of testing multiple organisms simultaneously. The applicants' first milestone deals with developing four prototype FONA biosensor detection devices for internal testing and validation.

The second milestone includes completion of internal testing and scientific validation of the technology. A commercial beta version of the instrument will be placed with strategic partners GAP/CRA to compare the FONA biosensor's performance to that of current 'real-time PCR and plating tests' for coliform in wastewater.

The third milestone includes the completion of external validation of the technology by GAP/CRA. Feedback and lessons from GAP will be incorporated into the final commercial laboratory unit. Further contributions from GAP/CRA will include collaboration on the standardization of FONA water testing protocols and input into the final design of the test kits. Through these exercises the scientists will gain valuable understanding of the training requirements for the lab staff that will be using the FONA technology. The last element of the third milestone is the commercial release of laboratory units.

During the project, efforts will be placed on designs for transportability of the detector and miniaturization of the sensor. Additional challenges relating to this project include making the instrument user friendly for less skilled personnel who are not necessarily situated in labs, and trying to develop a platform that can be used for a multitude of purposes including food and water safety, animal health and antimicrobial resistance.

DM20 - Project Title: Detection of *Campylobacter jejuni* by fluorescent *in situ* hybridization

Project Leader : Dr. Heidi Schraft, Department of Biology, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1
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Project Duration: May, 2002 - April, 2004

Abstract:

Campylobacter jejuni is the leading cause of foodborne disease in North America and it is estimated that over 2.5 million cases occur each year in the US. These cases lead not only to acute symptoms of severe diarrhea and fever, but also to a higher incidence of an autoimmune disorder, the Guillain-Barre syndrome. In addition to medical cost, association of the pathogen with poultry also causes potential losses to the poultry industry due to consumer concerns regarding the safety of poultry products.

Hazard Analysis Critical Control Point (HACCP) systems require verification procedures that include confirmation of the presence of pathogens in finished product and processing environments. *C. jejuni* is a microaerophilic organism that requires specialized culture conditions for growth. This involves the use of special gaseous atmospheres and culture supplements. Thus, only a few specialized laboratories can currently analyze food and environmental samples for *C. jejuni* and testing tends to be very expensive. This significantly impedes monitoring and surveillance of *C. jejuni* in poultry production.

This project will use the molecular biology approach known as fluorescent *in situ* hybridization to develop a rapid, culture independent procedure for the detection of *C. jejuni* in poultry and in environmental samples from poultry processing environments. This approach will overcome the difficulties associated with culturing while still being specific for viable *C. jejuni*. The work will be completed in three milestones: first, the specificity of an existing fluorescent *in situ* hybridization (FISH) protocol will be confirmed using a wide range of *C. jejuni* isolates and non-*Campylobacter* isolates. Second, a buoyant centrifugation based procedure will be adopted which will allow to concentrate *C. jejuni* from large sample volumes and to separate microbial cells from particles that can interfere with FISH detection. Third, the newly established FISH protocol will be tested and validated with spiked and naturally contaminated poultry products and environmental samples from processing environments.

The results of this study will support poultry producers and processors in their ongoing efforts of reducing prevalence of the pathogen in the production chain and provide OMAF with a cost-effective procedure for surveillance and enforcement programs.

DM21 - Project Title: Development of an analytical method for the confirmation of sulfonamides in animal tissues

Project Leader: Louise Spilsbury, Laboratory Services Division, University of Guelph, Guelph ON N1G 8J7
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Project Duration: May, 2001 - April, 2003

Abstract:

Sulfonamide antimicrobials have been used in livestock for many years, both for the treatment of bacterial infections and for growth promotion. The risk of residues in animal tissues is very real, especially in young animals such as barbecue hogs that may not have been allocated a long enough withdrawal period before slaughter.

The Ontario Ministry of Agriculture and Food has extensive surveillance programs for animals slaughtered at provincial abattoirs. These programs involve taking tissue samples directly from suspect carcasses for analysis, or, in the case of market and barbecue hogs, using a urine screen in order to more accurately target carcasses requiring further testing.

The objective of this project is to develop and validate an HPLC method for the determination of sulfonamide residues in animal tissues. The method will enhance regulatory testing capabilities through its ability to detect and quantify up to eight sulfonamides in animal tissues, compared with only three for the currently-used method.

The work to date has been conducted on swine muscle and liver samples. Sulfamethoxypyridazine is added to the tissue as an internal standard. Sample clean-up begins with homogenization with sodium sulfate and ethyl acetate, followed by centrifugation and removal of the solvent by evaporation. The residue is redissolved in 50% ethyl acetate in hexane, and loaded on to solid phase extraction columns (Bakerbond C18). The sulfonamides are eluted with 80% pH 7.2 phosphate buffer 20% acetonitrile. An aliquot is derivatized with fluorescamine and 25 µL injected on to the HPLC. Separation is by reverse-phase HPLC (Symmetry C18 column, gradient mobile phase of acetate buffer pH 5.2 acetonitrile). The calibration curve for most of the sulfonamides ranges from 0.035 to 0.2 ppm ($r^2 = 0.999$). Recovery levels for the eight sulfonamides in muscle range from 63% for sulfathiazole to 89% for sulfamethazine. With the internal standard correcting for variations in recovery, the mean accuracy for maximum residue levels (MRL) of all sulfonamides in muscle $99.8\% \pm 2.9\%$ ($n = 5$).

Once validated, the method will be compared with Laboratory Services' existing CFIA-accredited TLC method. The new method offers several advantages. It uses no halogenated solvents, which are toxic and expensive to dispose of. It removes the need to maintain an aging and obsolete scanning densitometer that has limited application. The Ontario food system will benefit from the wider range of sulfonamide residues capable of being detected and quantified in the meat of food animals. It is anticipated that the method will be readily adaptable to other matrices, such as meat-and-bone meal, and feed.

DM22- Project Title: Development of an analytical method for the quantitative detection of cephalapirin residues in bovine milk

Project Leader: Louise Spilsbury, Laboratory Services, University of Guelph, Guelph, ON N1G 2W1
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Collaborating Researcher(s): Dr. John Lynch, University of Guelph and Dr. Bruce McNab, Ontario Ministry of Agriculture and Food

Project Duration: June, 1998 - May, 2001

Abstract:

Cephapirin is a semi-synthetic cephalosporin which is used as an intramammary infusion for treating bovine mastitis. In Canada, two formulations are available. One, a cephapirin benzathine preparation, is used to treat the dry cow. Because it is designed to provide bactericidal levels of cephapirin for a prolonged duration, it should not be used within 30 days of calving. Withdrawal time after calving is 84 hours. The other, a cephapirin sodium preparation, is used in the lactating cow and has a withdrawal time of 96 hours.

There is some concern that even when proper withdrawal times are adhered to, residues may be incurred. In a study on the persistence of residues in milk, Seymour et al. observed that 40% of cows treated with cephapirin sodium showed the presence of residues after the recommended withholding time of 96 hours.

Cephapirin residues above the maximum residue limit (MRL) of 0.02 ppm are not allowed in milk. A method capable of quantifying cephalosporins at a level of 0.005 was developed for raw bovine milk. Cephalothin is added to the milk as an internal standard. Sample clean-up involves deproteinization with aqueous zinc acetate-phosphotungstic acid-acetic acid solution, followed by solid phase extraction (un-encapped C₁₈, United Chemical Technologies). The cephalosporins are eluted from the column with acetonitrile. After removal of the acetonitrile by evaporation under nitrogen, residues are re-dissolved in water and injected on to the HPLC. Separation is by reversed-phase HPLC (Symmetryshield RP18 column, gradient mobile phase of phosphate buffer pH 7.2/acetonitrile, and UV detection at 260nm). The calibration curve ranged from 0.005 to 0.4 ppm ($r^2 = 0.999$). A sensitivity of 1 ng on-column was achieved, equivalent to 0.005 ppm in milk. The inter-assay variability for samples fortified at the MRL was 6.3%. The method worked well in bulk samples from the Ontario Raw Milk Quality surveillance program. Comparisons were made with the *B. stearothermophilus* disk assay and with immunological screening tests. Confirmation by LC-MS was carried out on selected samples.

DM23 - Project Title: DNA fingerprinting analysis of *E. coli* using repetitive element and toxin specific gene amplification to differentiate pollution sources in the environment

Project Leader: Michele Van Dyke, GAP EnviroMicrobial Services, 1020 Hargrrieve Road, Unit 14, London, ON N6L 1P5
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Collaborating Researcher Establishments: Ontario Clean Water Agency, Upper Thames River Conservation Authority, Conestoga-Rovers & Associates

Abstract:

Controlling the introduction of fecal waste in surface and groundwater is an important issue in Ontario. The presence of pathogenic bacteria in potable, agricultural and recreational water supplies can have grave consequences, and the incidence of these occurrences appears to be increasing in recent years. To effectively control inappropriate releases of fecal waste to the environment, methods to determine the point source of origin must be available. This research project attempts to address this apparent lack of available methods.

Escherichia coli is a useful indicator of fecal contamination, and we used this strain together with methods for subspecies analysis. If individual subspecies of *E. coli* can be traced to a specific type of animal host, the analysis can then be used to differentiate between point sources of origin of fecal waste. Molecular methods are able to distinguish between closely related bacteria by producing DNA patterns or fingerprints that are specific for individual strains. The method selected for this analysis was DNA amplification of repetitive-element sequences (rep-PCR).

Strains were amplified using 3 different rep-PCR primers sets, including BOX, REP and ERIC. Phylogenetic analysis was then performed on composite data sets of the fingerprint patterns. The amplification conditions provided reproducible and reliable banding patterns for each strain. Results of the *E. coli* analysis by individual host animals did not separate well, but when host animals were grouped into 4 main categories, including farm animals, avian, wildlife, and human, the categories grouped better, but with less host specificity. Category separation values ranged from 76% for farm animals to 53% for human. Data was also analyzed by selecting representative isolates from strains that gave the same pattern from an individual animal. As expected, this analysis lowered the category separation values, ranging from 59% for farm animals to 12% for wildlife.

In addition to *E. coli*, strains of *Enterococcus faecium* were isolated. It has been suggested that *Enterococcus* strains may be a better indicator of fecal source contamination than *E. coli*. Cluster analysis of *E. faecium* isolates showed that the separation of farm animals versus avian was very successful. However, the database of *Enterococcus* is small compared to *E. coli*, therefore we are continuing to add *Enterococcus* strains from a variety of animals to the database to determine if this species provides more reliable group separations values than *E. coli*.

The *E. coli* samples for this study were obtained from a large geographical area, and it is possible that this may be the cause of poor grouping of *E. coli* isolates with their host. Currently, we are assessing whether rep-PCR fingerprinting can track the source of *E. coli* strains within small, defined environments. Positive results from this work will show potential for this type of analysis in sites where suspected sources are known, and that have full access for sample collection.

Risk Assessment Category (RA)

RA01 - Project Title: Survival and release of fecal bacteria from rural stream sediments

Project Leader(s): Dr. Doug Joy, School of Engineering, University of Guelph, Guelph, ON N1G 2W1
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Project Duration: May, 2002 - April, 2003

Abstract:

The primary objective of this proposed project is to quantitatively assess the impact of stream sediment fecal bacteria reservoirs on the bacteriological quality of surface water systems. Fecal bacteria survival and transport patterns in surface water systems are currently poorly understood and poorly represented in watershed-scale surface water quality models. Several studies have indicated that fecal bacteria can survive for extended periods of time in stream sediments. Extended bacterial survival makes it difficult to precisely identify sources of fecal pollution. In this study, bacterial survival and transport will be quantitatively evaluated, *in-situ*, using a tracer bacterium. Within 4-6 rural streams, a 1 x 1m section of bottom sediment will be seeded with nalidixic acid resistant (NAR) *E.coli*. The release of the seed bacteria into the water column will be monitored and correlated with hydrologic and environmental variables. The die-off and growth dynamics of the seed bacteria within the stream sediment will also be assessed. This project will provide valuable information that will aid in the development of representative water quality simulation models. This, in turn, will allow researchers and regulatory agencies to focus their efforts on addressing the primary sources of bacterial pollution in surface water systems.

RA02 - Project Title: Fuzzy and statistical techniques for food safety risk characterization and valuation

Project Leader: Dr. V. Davidson, School of Engineering, University of Guelph, Guelph ON N1G 2W1
Tel: 519-824-4120 x 4367 Email: vdavidso@uoguelph.ca

Project Duration: May, 2000 - April, 2002

Abstract:

The objective of food safety risk assessment is to quantify levels of risk to consumers in order to evaluate the level of risk against costs of risk reduction as well as competing risks. Risk assessment includes hazard characterization (i.e. defining adverse impacts of food contaminants on human health) as well as valuation (i.e. assigning values to predicted impacts). In applying risk assessment methods to food systems, there is a need to deal with uncertainties in terms of system parameters and biological effects. Dr. Bruce McNab (Risk Management Unit, Ontario Ministry of Agriculture and Food) has developed a general framework for microbial food safety risk assessment. At present, this framework uses statistical methods to deal with uncertainties related to microbial growth and dose-response estimates.

Fuzzy mathematics is another approach to dealing with uncertainties. It offers tools for describing variability in model parameters (fuzzy set theory) as well as inferencing techniques (fuzzy logic) that are useful for decision-making and risk evaluation. Fuzzy techniques are complementary to statistical tools to describe uncertainty.

Simulations were run to compare fuzzy and probability-based methods for two risk assessment scenarios: estimation of biological impact (McNab general framework) and survival of *Campylobacter jejuni* through five poultry processing operations. In Monte Carlo simulations, the propagation of uncertainty is limited by the probability-based sampling of parameter values over a large number of iterations. Fuzzy calculations are done in a single step, assuming that all combinations are possible, and the interval defining possible values can be very large relative to probability-based sampling. In general the fuzzy results were more conservative for both risk assessment scenarios. Conservative estimation is a desirable characteristic in early stage risk assessment when there is usually limited data to define credible probability distributions for all system parameters. Fuzzy simulation techniques can be used at the outset and probability-based measures can be incorporated, as more reliable information becomes available.

A cross-disciplinary group with interests in quantitative risk assessment has recently received NSERC funding from the Strategic Grants Program ("Microbial Food Safety - Integrated Modelling Techniques for "Farm-to-fork" Risk Assessment"). The group has been working to develop a general computational framework for risk assessment that integrates fuzzy and statistical methods to define uncertainty. The group includes expertise from several departments at the University of Guelph, Ontario Ministry of Agriculture and Food, Health Canada and Agriculture and Agri-Food Canada.

Microbial foodborne illnesses are a concern to Ontario food processors as well as consumers. As the preparation and distribution chain becomes longer and more complex, it is important to develop quantitative risk assessment tools that can be used to design and implement risk management strategies. In December of 2000, the Ontario Expert Sub-committee on Meat Products and Processing identified microbial risk assessment tools as its number one research priority.

RA03 - Project Title: HACCP tools and integrated models for microbial risk assessment

Project Leader: Dr. V. Davidson, School of Engineering, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researcher: Dr. S. Yang, School of Engineering (syang@uoguelph.ca)

Project Duration: May, 2002 - April, 2004

Abstract:

The assessment of microbial risks in food systems is a significant issue in processing operations as well as international trade. A group of researchers (University of Guelph, Brock University, Agriculture and Agri-food Canada, Health Canada and Ontario Ministry of Agriculture and Food) are collaborating to develop integrated modelling techniques for microbial risk assessment. The goal is to develop techniques that can be used for early stage research and model structures that can be expanded to include more sophisticated tools (e.g. Monte Carlo simulations) as knowledge of the systems becomes more reliable and complete. The long-term goal is to develop risk assessment models that can be used to predict economic impacts of foodborne diseases (health costs, lost productivity) and to assess impacts of risk reduction strategies. This OMAF-sponsored project will complement the modelling activities being conducted for an NSERC strategic grant. The focus in the OMAF project is to use the risk models to develop practical HACCP tools for processors.

The fundamental modelling of processes and systems needs to be validated with data from typical industrial operations. The validated results will be used to develop and monitor HACCP systems. Both areas will be investigated through discussions and information sharing with Ontario food processors.

Integrated risk assessment models are useful in developing effective practices to ensure food safety. Integrated models include qualitative information (such as experts' opinions) and quantitative information (such as probability distributions to describe variability of process inputs). The goal is to develop practical tools from these models that can be used at the level of day-to-day HACCP procedures. These would be useful for Ontario processors, particularly sectors that are facing mandatory HACCP procedures.

RA04 - Project Title: Effect of pesticides in recycled water used in mushroom compost preparation on mushroom production and quality

Project Leader: Dr. Greg Duns, Laboratory Services, University of Guelph, 95 Stone Road West, Guelph, ON N1H 8J7 Tel: 519-767-6211 Email: gduns@lsd.uoguelph.ca and Dr. Danny Lee Rinker, Department of Plant Agriculture, University of Guelph, 4840 Victoria Avenue, Box 7000 Vineland Station, ON L0R 2E0 Tel: 905-562-4141 x132 Email: drinker@uoguelph.ca

Collaborating Researchers: Dr. Brian Ripley, Linda Lissemore, Pam Leishman, Laboratory Services, University of Guelph

Project Duration: January, 2000 - March, 2002

Abstract:

This research addresses important issues concerning the relationship between residual pesticides in the recycled water used in mushroom compost preparation, and mushrooms produced on a compost substrate exposed to this recycled water containing pesticides. Specifically, this research is designed to:

- determine if pesticide residues accumulate in tissues of mushrooms produced on compost prepared by using pesticide-containing recycled water;
- investigate the effects of pesticides from recycled water on the quality and production of mushrooms grown on compost prepared by using this recycled water;
- study the ability of the composting process to degrade pesticides contained in the recycled water; and
- examine the impact on the environment of pesticide residues in recycled water from mushroom farms.

The results of this research will ensure that Ontario mushroom production guidelines do not result in violations of maximum residue limits under the *Canadian Food and Drug Act*, and will ensure that Ontario mushroom production practices do not result in environmental contamination through water run-off. In addition, as composting is becoming an increasingly important method for treating organic waste products, determining the relationship between pesticides and composting will enhance general knowledge of environmentally sound composting practices.

RA05 - Project Title: Quantifying water quality impacts of livestock farms and other rural land uses

Project Leader: Ron Fleming, Ridgetown College, University of Guelph, 120 Main Street East, Ridgetown, ON N0P 2C0
Tel: 519-674-1612 Email: rfleming@ridgetown.uoguelph.ca

Project Duration: May, 1999 - April, 2003

Abstract:

This study will attempt to put into perspective the various sources of water contaminants in a watershed. It will examine microbiological indicators, such as bacteria, *Cryptosporidium* and *Giardia*. It will establish typical impacts of a variety of agricultural practices. Factors such as type of tillage, crop fertility program, livestock manure spreading, and type of crop grown all can affect water quality. The study will go further, though, and attempt to determine the potential impacts of other land uses in the watersheds. Often, agriculture is assumed to be the largest contributor of contaminants in a rural watershed, since it has the largest land area. Other impacts are not well documented: for example, illegal connections of septic systems to drainage tiles, sewage treatment plant discharges of raw sewage, and wildlife impacts.

Manure and drainage water samples will be analyzed for levels of *Cryptosporidium* and *Giardia*. This will help show the potential for manure management practices to impact levels of these contaminants in surface water, compared to other typical land uses in a watershed.

A second part of the study will complete a project already underway. This deals with the manure volumes on swine farms using wet dry feeders. This feeding system reduces water wasted in a pig barn. This can have a major impact on farmers using nutrient management planning to determine manure spreading rates. This, in turn, can have an impact on the potential for contamination of surface water or groundwater. Several farms are being monitored to determine manure production rates and manure nutrient concentrations under the two management practices used on most swine farms. Water needs of the pigs are being measured.

RA06 - Project Title: The effect of nutrient availability on biogeochemical processes affecting the transport of *Escherichia coli* in soils

Project Leader: Dr. Danielle Fortin, Dept. of Earth Sciences, University of Ottawa, 140 Loius Pasteur, Ottawa, ON
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Abstract:

Escherichia coli is a pathogenic bacterial species associated with manure. Because large quantities of manure are produced by the pork and cattle industries, and because the manure is susceptible to leaching by rain and surface water, *E. coli* is a common groundwater contaminant in rural Ontario. An ability to predict the speed and extent of *E. coli* transport through soils is therefore essential to evaluate the environmental risks associated with pork and cattle production in Ontario, and, where required, to facilitate environmental remediation.

In this investigation, batch experiments will be conducted to determine the degree of *E. coli* adhesion to soil minerals as nutrient availability, solution pH and soil mineralogy are independently varied. We predict that nutrient limitation will affect cell size, surface electric size, hydrophobicity and metabolic state. Nutrient concentrations will also affect solution composition and ionic strength. We expect that these changes in cell morphology, reactivity and solution composition will affect the extent to which the cells adhere to different mineral surfaces.

The major deliverable of this project will be a predictive model that can be applied to determine the degree of bacteria-mineral adhesion in a variety of different biogeochemical regimes. This can be used to evaluate the environmental risks associated with manure leaching. The use of this sort of predictive tool will not only reduce the risk of environmental contamination in Ontario, but it will also provide an economic benefit, in that costs associated with site remediation will be lessened.

RA07 - Project Title: Surveillance of Ontario pig farms for diseases of public health significance

Project Leader: Dr. Robert Friendship, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. C. Dewey, Department of Population Medicine, University of Guelph; Dr. S. McEwen, Department of Population Medicine, University of Guelph; Dr. C. Gyles, Department of Pathobiology, University of Guelph

Project Duration: July, 2001 - July, 2003

Abstract:

A sample of 100 pig farms, proportionately weighted by herd size and regional distribution has been selected randomly from a list of producers supplied by Ontario Pork. Blood samples have been taken from 30 sows and 30 finishing pigs, and fecal samples have been collected from 15 of the finishing pigs supplying blood samples. A short survey has been completed at the time of the farm visit, providing information about production and drug use. Samples have been processed and stored in a manner allowing future tests to be readily conducted.

Fecal samples are being cultured for *Salmonella*, *E. coli* including O157:H7, and *Yersinia enterocolitica*. Antibiotic resistance patterns of *Salmonella* and non-pathogenic *E. coli* will be assessed and compared with farm drug use data. Serum will be analyzed for Influenza, and *Toxoplasma gondii* antibodies. Sampling and testing will be repeated 12 months later.

The prevalence at the farm level of important food borne pathogens will be determined and risk factors examined. A network of producers, veterinarians, diagnostic lab workers, and researchers will be established and a resource of stored sera and feces will be created and made available for additional studies of disease surveillance. New tests will be developed and validated.

Highlights of the work to date include the isolation of *E. coli* O157:H7 from the feces of pigs on several pig farms in Ontario, the first study in North America to show that healthy pigs may carry this potential human pathogen. *Salmonella* was cultured from about 10% of the herds. Monitoring the prevalence of *Salmonella* by culture and by serology was studied. It appears that commercially available ELISA tests measuring antibody titres can be used to screen for herds where *Salmonella* may be a problem in that a strong correlation between culture and serology results was observed. Approximately 80% of Ontario pig farms tested were found to have antibody evidence of exposure to swine influenza virus H1N1, with negative farms being almost exclusively located in Eastern Ontario. Enteric protozoa with food safety implications including *Balantidium*, *Giardia* and *Cryptosporidia* are being monitored and some herds have been identified as positive. Testing of *Yersinia* and *Toxoplasma* is being conducted. We will be attempting to assess what factors are associated with the presence of one or more of these potential pathogens and whether a herd with one is more likely to have others as well.

The anticipated benefit is that at the completion of the study the farm prevalence of the important food safety pathogens will be known and the major risk factors associated with the presence or absence of these pathogens can be identified. It is hoped that steps may then be taken to eliminate or minimize the prevalence of these organisms on pig farms and therefore reduce the risk of pathogens being introduced to the abattoir.

RA08 - Project Title: Unregulated metals in biosolids: risks to the environment, plant and human health

Project Leader: Dr Bev Hale, Department of Land Resource Science, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2000 - April, 2006.

Abstract:

Public concern about the application of sewage biosolids to agricultural land is growing as reduced incineration and landfilling of these wastes increases their use in soil amendment. The application of biosolids to agricultural land supplies nutrients (N and P) to crops, organic matter to soils and provides disposal of waste materials. There are guidelines for the rate of biosolid application, the soil conditions under which application is permitted and waiting periods between application and crop harvest utilization. These guidelines minimize the transfer of pathogens and various metals present in the biosolid material to the food chain, and prevent the accumulation of metals in soils. Risk assessments (RA's) for land application of many metals in biosolids are relatively well developed. However, RA's for other contaminants in biosolids are weak, specifically for organics, pathogens and unregulated metals. Quantification of unregulated metals in Ontario biosolids, and plant uptake of these metals specific to Ontario soils and crops, will contribute to risk management for biosolids application to agricultural land. Current guidelines for biosolids application must be demonstrably protecting food and soil quality, and human health. For the 'known' metals (Cu, Zn, Co, Pb, Cd etc.), there is some knowledge of the relationships between soil and plant concentrations. However, other metals are unregulated in sewage biosolids, thallium, tin, beryllium, silver and antimony are emerging as elements of concern for soil, plant and human health, and very little is known of their fate and effects after application to soil. This research project will survey biosolids from Ontario sewage treatment plants, to determine the concentrations of these unregulated metals that would be added to agricultural soils. Re-analysis of archived soil and plant samples from biosolids-amended field plots will demonstrate whether there have been measurable additions of these metals to agricultural systems, under field conditions. Bioavailability of these metals to plants grown in soils, and to mammals from food will be evaluated, to determine the potential for transfer through the food chain.

The project objectives include:

1. Determine the potential for addition of thallium, tin, antimony, beryllium and silver to agricultural soils through biosolids application.
2. Determine the potential for plant uptake of these five metals from biosolids amended soils.
3. Evaluate the potential for transfer of these five metals, when plant-accumulated, to human intestinal cells.

There was no detectable metal accumulation by either Swiss chard, or beet root when grown on sandy loam or clay loam soils, recently supplemented with metal-containing sewage biosolids, applied at rates up to double the current allowable maximum, relative to control plots receiving no sewage biosolids. Sewage biosolids are rarely applied to vegetable crops in Ontario because of the required 12 months waiting time between waste application and harvest. This waiting time is designed to prevent the transfer of pathogens from the biosolids to consumers of the produce. Pelletizing of biosolids which reduces or eliminates pathogens removes this barrier to their use in vegetable production, so other risks of biosolids application to crop health, such as accumulation of non-nutritional metals, need to be addressed.

RA09 - Project Title: Characterization of *E. coli* O157:H7 from pigs in Ontario

Project Leader: Dr. C.L. Gyles, Department of Pathobiology, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. RM Friendship (Department of Population Medicine)

Project Duration: May 2002 - April 2004

Abstract:

Although pigs have never been implicated in any human infections with *E. coli* O157:H7 in North America, it is important to know whether pigs do carry these organisms. *E. coli* belonging to serogroup O157 have long been known to be shed by pigs in their feces, but these bacteria are different from *E. coli* O157:H7 and do not constitute a risk for humans. Recently, it has been demonstrated that *E. coli* O157:H7 are present in the feces of some pigs in some herds in Ontario. The purpose of this project is to continue that study. The study will determine whether *E. coli* O157:H7 bacteria persist on pig farms throughout the year, to assess the numbers of organisms that are shed by pigs, and to characterize porcine *E. coli* O157:H7 isolates to determine whether they are different from those implicated in human disease. In particular, because it has been noted that some of the *E. coli* O157:H7 isolated from pigs were nontoxigenic, the study will investigate the stability of toxin genes in porcine *E. coli* O157:H7 isolates. The study will also include environmental sampling on positive farms and will try to determine whether positive farms have characteristics that are different from negative farms.

RA10 - Project Title: *E. coli* O157:H7 in veal calves in Ontario

Project Leader: Dr. C.L. Gyles, Department of Pathobiology, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2002 - April, 2004

Abstract:

The major objective of the research is to determine why veal calves appear to resist colonization by *E. coli* O157:H7. This study will also identify sites in the intestine of conventional calves that are colonized by this organism. Sensitive detection techniques will first be used to confirm that there is a low prevalence of *E. coli* O157:H7 in veal calves in Ontario. This will be followed by experimental techniques to determine the reason for this low prevalence, which contrasts with a high prevalence in both beef and dairy cattle, and to determine the areas of the intestine which harbour *E. coli* O157:H7. Specifically this research will explore the roles of diet, rumen development, and antibiotics in the limitation of *E. coli* O157:H7 in veal calves. These studies will assist in the development of methods for controlling colonization of beef cattle by *E. coli* O157:H7.

RA11 Project Title: Investigation of the prevalence, diagnosis and management of Johne's disease in Ontario dairy herds

Project Leader: Dr. D. Kelton, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. T. Duffield (Population Medicine), Dr. K. Leslie (Population Medicine), Dr. K. Lissemore (Population Medicine)

Project Duration: May 2002 - April 2006

Abstract:

Johne's disease, a chronic debilitating bacterial enteritis of cattle caused by *Mycobacterium paratuberculosis*, has long been considered a production limiting disease of moderate economic consequence to most North American dairy herds. Recently interest in the disease has increased dramatically as evidence accumulates for the potential link between Johne's disease and Crohn's disease in humans. A recent survey of a purposive sample of Ontario dairy farms indicates that the disease is present in approximately 10% of herds. There is a need to confirm this prevalence estimate, at both the herd and cow levels. Furthermore, there is a need to further elucidate the herd level risk factors that predispose herds to Johne's disease. This project will accomplish both of these objectives as well as to investigate the usefulness of a new milk ELISA antibody test that can very conveniently be applied to preserved milk samples collected by dairy herd recording agencies for routine composition testing. Finally, it has been suggested that the inclusion of ionophores in the ration of dairy cattle may decrease the fecal shedding of viable *Mycobacterium paratuberculosis* organisms. This project will investigate this possibility through the administration of a controlled clinical field trial.

RA12 Project Title: Assessing the incidence of antimicrobial resistant *E. coli* and *Salmonella* bacterial isolates in cull cows from Ontario free stall dairy herds

Project Leader: Dr. David Kelton, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. K. Lissemore, Dr. S. Martin, Dr. S. McEwen, University of Guelph; Dr. R. Reid-Smith, Health Canada

Project Duration: May, 2001 - March, 2003

Abstract:

In recent years, the development of resistant bacteria associated with the use of antimicrobial drugs in food producing animals has emerged as a major food safety concern. The raw milk products and meat from cull dairy cows represent a potential risk in this regard. This study will focus on the latter. Given that dairy cull market cattle contribute significantly to overall beef consumed it is important to determine the incidence of antimicrobial resistant bacterial isolates in these animals.

The objective of this project is to determine the incidence of antimicrobial resistant *E. coli* and *Salmonella* spp. in cull dairy cows entering the food system as beef using an incidence density case-control observational study design. A second objective is to identify key aspects of facility design and cow management that are associated with herd level antimicrobial drug use and the incidence of resistance development.

Surveys assessing various aspects of antimicrobial use were mailed to all producers with free-stall dairy barns and all livestock veterinarians in the province of Ontario. Additionally, 25 free-stall herds are currently enrolled in a longitudinal study in which producers track individual and herd level drug use for a one year period using a paper-based treatment record and a *garbage can auditing* (GCA) system. Cull cows from the study herds will be assessed with regard to previous exposure to antimicrobial drugs and fecal samples will be collected and cultured for *E. coli* and *Salmonella* isolates will be typed. The incidence of resistant isolates in cull cows (cases) will be determined and compared to the incidence in cows remaining in the herd (controls). Cow and herd level data will be collected to be included as predictor variables in a model that will assess the association of the incidence of antimicrobial drug resistant *E. coli* and *Salmonella* in cull cows with specific risk factors.

Data collection and fecal sample submission and testing are ongoing through the calendar year 2002 study period.

The survey response rates were 51% and 47% for the dairy producers and veterinarians respectively. Both groups ranked the reasons for antimicrobial use in lactating dairy cattle: 1-mastitis, 2-uterine vaginal infection, 3-lameness, 4-respiratory infections. Ceftriaxone, penicillin, trimethoprim-sulfadoxine and oxytetracycline were the most frequently veterinarian dispensed injectable antimicrobials, while a novoxbioicin-streptomycin-penicillin-G-polymyxinB combination product and cephalixin were the most frequently dispensed intramammary antimicrobials. These findings were confirmed by study herd drug use data. In two clinical case questions the frequency of systemic antimicrobial therapy, vs. local or non-antimicrobial treatments, was 9% for mild mastitis in both groups but differed for a case of digital dermatitis with 13% of producers and 21% of veterinarians resorting to injectable preparations. Neither the GCA nor the treatment records revealed the use of quinolone antibiotics; ceftriaxone ranks third behind procaine penicillin, trimethoprim-sulfadoxine and tetracycline in frequency of use and grams of active antimicrobial drug used.

RA13 - Project Title: Prevention of microbial contamination in beef

Project Leader: Dr. S. McEwen, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2002 - April, 2006

Abstract:

Food safety is a major priority for the beef industry. There is urgent need to prevent beef contamination with human disease-causing bacteria. Beef has been the vehicle of several highly publicized outbreaks of human food borne disease such as *E. coli* O157:H7 and *Salmonella*. There is a demand from beef producers, processors and public health officials for the information and technology that underlie on-farm bacterial prevention strategies. An integrated beef safety risk management system from production through to consumption is needed to incrementally reduce pathogen load in meat and the risk to consumers. This includes a need for control strategies at the farm level. This need is not being met because of significant gaps in our knowledge, in particular of the population dynamics of human foodborne pathogens in cattle, and the efficacy of plausible interventions, such as vaccination. The main purpose of this project is to undertake biologically-based research involving epidemiological, experimental and risk assessment modelling studies needed for the development of beef safety risk management strategies.

RA14 - Project Title: Antimicrobial resistance in selected bacteria of beef cattle and associations with on-farm antimicrobial use

Project Leader: Dr. Scott McEwen, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
Tel: 519-824-4120 x 4751 Email: smcewen@uoguelph.ca

Collaborating Researchers: Richard Reid-Smith and Case Poppe, Laboratory for Foodborne Zoonoses, Health Canada

Project Duration: April, 2001 - October, 2002

Abstract:

This research will determine the associations between antimicrobial use on beef farms and antimicrobial resistance in enteric bacteria. It will also provide information for qualitative and quantitative risk assessments of the assessments of the attendant human health risks. The principal hypothesis is that antimicrobial use on beef farms selects for resistance in enteric bacteria, but the human health impact (measured in terms of morbidity and mortality) is small.

Field studies that will provide data on the use of antimicrobials in the Ontario beef industry are currently underway, and the same farms are now enrolling in a field study of antimicrobial resistance in selected enteric bacteria. Rates of antimicrobial use as well as under- and over-reporting will be calculated for various classes of animals (e.g. cows, calves, steers, etc.), stages of production (e.g. cow-calf, feedlot) and for various products (e.g. ionophores, injectable antibiotics, biologics, implants, etc.). Concurrently, this lab seeks to identify the prevalence of antimicrobial resistance in fecal bacteria of beef cattle on study farms. Fecal samples will be collected four times per farm, approximately three months apart. Additionally, two producers will be selected for an in-depth, intensive study to identify the incidence, persistence and spread of resistance among individual animals over time. The bacteria to be tested are *Escherichia coli* and *Salmonella* and resistance will be determined with respect to the antimicrobial drugs commonly used in cattle plus those of public health importance. Statistical and mathematical models will be used to assess incidence of infection (and recovery) and interrelationships among resistance and drug treatment, age of animal and other factors.

A collection of enteric bacteria from individual animals and pooled samples will be established. Funding from other sources will be sought for testing of genetic markers of antibiotic resistance. This information will be useful in studying the effects of antimicrobial use on the emergence and disappearance of resistant genes in individuals and groups.

RA15 - Project Title: Development of a framework for antimicrobial use recording among Ontario beef producers

Project Leader: Dr. Scott McEwen, Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1 Tel: 519-824-4120 x 4751 Fax: 519-763-3117 Email: smcewen@uoguelph.ca

Collaborating Researchers: Dr. Richard Reid Smith, Health Canada

Project Duration: August, 2000 - July, 2001

Abstract:

Cattle producers use antimicrobials to treat disease, prevent illness, and to improve feed efficiency or promote growth. Recently there has been much media attention and public concern about the use of antimicrobials in food-producing animals with respect to the transfer of antimicrobial resistance to humans. In Canada, details of drug use information in beef production has been lacking but is required to address consumer concerns that Canadian beef producers are proactively assessing the manner in which antimicrobials are used, in order to continue to produce a safe product.

The purpose of this research was to document antimicrobial use practices by Ontario feedlot and cow-calf producers and to quantify the amount of antimicrobials used in therapeutic and subtherapeutic concentrations. The effectiveness of producer recording was compared with an on-farm audit. Medication records were kept prior to this study on 11-16 feedlots and 3-13 cow-calf farms. Antimicrobials were used in-feed, water and by injection and a variety of drugs were used for various purposes. Some of the producers surveyed reported that accurate record keeping was difficult to maintain during outbreaks of disease. Our results indicate that there is significant under-reporting when records are compared with independent audits of antibiotic use on farms.

As this was a pilot study with volunteer participants, there is the potential for bias and thus results of this study cannot be accurately extended to encompass all beef producers across the entire province of Ontario. However, the information is useful as an initial indicator of the quantities and types of antimicrobials used, use practices, and as an indicator of the potential success of the implementation of a province-wide treatment recording system. The data collected will also be very useful in ongoing studies of antimicrobial use and resistance in beef cattle.

RA16 - Project Title: Occurrence of enteric pathogens and antimicrobial resistance patterns in selected retail poultry products and human cases in a southwestern Ontario county

Project Leader: Dr. Scott McEwen, Department of Population Medicine, University of Guelph University of Guelph, Guelph ON N1G 2W1 Tel: 519-824-4120 x 4751 Email: smcewen@uoguelph.ca

Collaborating Researchers: Dr. A. Deckert, Dr. R. Reid-Smith, Dr. R. Irwin, Dr. A. Valdivieso, Dr. K. Dore, Dr. P. Buck, Health Canada; Dr. F. Jamieson, Dr. B. Ciebin, Ontario Ministry of Health and Long Term Care

Program Term: May 2001 - March 2003

Abstract:

This project is an investigation into the occurrence of *Campylobacter* spp. in retail poultry in a county in southwestern Ontario, the occurrence of human campylobacteriosis, the risk factors associated with human campylobacteriosis, and the antimicrobial resistance patterns of poultry and human isolates.

The objectives of this study are: to investigate and compare the occurrence of susceptible and resistant *Campylobacter* spp. isolates in retail poultry and human cases of campylobacteriosis in a defined geographical area in Ontario; to investigate the risk factors for, and burden of illness resulting from, susceptible and resistant campylobacteriosis; and to investigate the temporal relationship between prevalence of *Campylobacter* contamination in poultry and the occurrence of campylobacteriosis.

The study will document and characterize the occurrence of susceptible and resistant strains of *Campylobacter* spp. in retail poultry over a one year period in a defined geographical area (subject county Health Unit). Poultry samples will be purchased at pre-determined intervals from retail outlets according to a defined sampling frame and protocol. *Campylobacter* isolates will be cultured and serotyped according to standard methodology and resistance tested using the E-test system. For the same time period, the stool samples of all diagnosed human cases of campylobacteriosis will be processed according to like protocols. Epidemiological data will be collected from cases and a randomly selected comparison group. The correlation between the resistance patterns observed in the poultry isolates and the human isolates, the temporal relationship between the level of *Campylobacter* isolated from retail poultry and the occurrence of campylobacteriosis, and the risk factors for the occurrence of susceptible and resistant campylobacteriosis will be analyzed.

The results contribute to the maintenance of Ontario's standards of food safety and preservation of public health by: providing preliminary Ontario-based prevalence and risk factor data; providing direction for future larger scale research needed to produce the type of representative data needed for meaningful assessments; provide preliminary data for the development of HACCP-based quality assurance programs; improve our understanding of the potential transmission of antimicrobial resistant strains of *Campylobacter* via the food-chain; and provide the basis for further initiatives including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant strains of *Campylobacter* in retail poultry and the development of an active surveillance system for antimicrobial resistant foodborne pathogens in Ontario.

The study is a collaborative effort among the Department of Population Medicine (University of Guelph), the Laboratory for Foodborne Zoonoses (Health Canada), the Division of Enteric, Foodborne and Waterborne Diseases (Health Canada), the Central Public Health Laboratory (Ontario Ministry of Health and Long Term Care) and the subject Health Unit.

RA17 - Project Title: Quantifying preferential flow and recharge at the field scale: the first step in characterizing the nature and timing of pathogen transport to groundwater

Project Leader: Dr. Gary Parkin, Department of Land Resource Science, University of Guelph, Guelph, ON N1G 2W1
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Collaboration Researchers: Richard Heck, Murray Brown, David Elrick

Project Duration: October, 2001 - October, 2004

Abstract:

In a recent survey of water wells in rural Ontario, it was discovered that approximately one-third was contaminated with coliform bacteria. In fact, the number of wells with fecal coliform bacterial counts over the guidelines may have almost doubled in the past 45 years. A recent Ontario study has shown that water flow through cracks and macropores in soil can transport bacteria 100 meters or more from sources such as septic systems and manure storage facilities. This research project will quantify the amount of water that flows preferentially through soil on an annual basis. It will document the seasons during the year when most preferential flow occurs and how the amount of deep drainage is affected by position in a sloping landscape and distance to tile drains.

Innovative methods will be used to measure the quantity of deep drainage that occurs through macropores such as cracks and worm burrows in the soil. Automatic tile flow and groundwater level monitors and water sampling systems will be used to determine how much water reaches tile drains or the water table preferentially during rain and snow melt events throughout the year. It will also help to define during which season(s) drinking water wells will be most susceptible to contamination from bacteria. Results from this research will assist in determining where best on the landscape to install instruments to measure the field-average recharge and potential leaching losses of contaminants from agricultural sources. This is invaluable information for groundwater protection schemes that can be of significant economic benefit to Ontario.

To date this research has determined that most deep drainage occurs in the late winter and spring seasons. However, preferential flow can occur in mid-summer.

RA18 - Project Title: Epidemiology and impact of *Cryptosporidium* on Ontario dairy farms

Project Leader: Dr. A. Peregrine, Department of Pathobiology, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. T. Duffield (Population Medicine), K. Leslie (Population Medicine)

Project Duration: May 2002 - April 2006

Abstract:

Cryptosporidiosis is a diarrheal disease caused by a coccidian parasite (*Cryptosporidium parvum*) that infects humans and farm animals, particularly calves. Infection is via the ingestion of oocysts. In calves, signs of illness include lethargy, fever, diarrhea and dehydration, which may lead to mortality. In humans, cryptosporidiosis is usually a self-limiting disease. However, the parasite may cause deaths in immunodeficient individuals. Waterborne oocysts can survive freezing and chemical disinfection, and are sufficiently small that they may not be removed by filtration systems. Outbreaks of cryptosporidiosis in people are therefore frequently associated with waterborne transmission, as occurred in 2001 in North Battleford, Saskatchewan.

Cryptosporidium parvum is commonly found in dairy calves in the first month of life and can play an important role in neonatal calf diarrhea. However, the prevalence and impact of infections in Ontario calves have not been determined. Furthermore, the risk factors for infection and disease due to *C. parvum* have not been identified. Such information is required to improve control of *C. parvum* as there are currently no drugs or vaccines for this parasite in Canada. Finally, the role of cattle as a source of parasites for human cases of cryptosporidiosis in Ontario has yet to be determined.

This project will use a large number of cooperating dairy herds in Ontario to obtain data on the prevalence of *C. parvum* in calves and to determine the extent to which infections are associated with disease. In initial work carried out from May to August of 2002, fifty participating dairy farms with a diarrhea problem were visited every second week for an observational study. During this

period, fecal samples were collected from all calves aged 7 to 21 days and examined for oocysts of *C. parvum*. In total, fecal samples were collected from 777 calves. The resultant data indicated that 76% of herds had calves infected with *C. parvum* during the study period. Furthermore, the calf-level prevalence of infection was 33.5%.

In the future, a 2-year prospective study will be carried out on Ontario dairy farms (1) to determine the influence of *C. parvum* on calf viability and growth rates, (2) to identify herd-level risk factors for *C. parvum* infection and cryptosporidiosis, (3) to identify calf-level risk factors for *C. parvum* infection and cryptosporidiosis, (4) to determine the role of adult cattle in the epidemiology of *C. parvum*, and (5) to compare the performance of diagnostic tests for *C. parvum* in cattle. A new formulation of halofuginone lactate (Halocur®) will also be assessed for its prophylactic potential in dairy calves as preliminary data indicate that the drug has significant activity against *C. parvum*. Finally, isolates of *C. parvum* will be obtained from cattle and people across Ontario and genotyped using a polymerase chain reaction method to determine the extent to which cattle are a potential source of *C. parvum* infections in people.

The information obtained from this work will lead to improved methods for controlling the prevalence and impact of *C. parvum* on dairy farms. In addition, the data will help rationalise programs for manure and environment management on farms.

RA19 - Project Title: Role of biofilm formation in the persistence of *Listeria monocytogenes* in food processing

Project Leader: Dr. H. Schraft, Department of Biology, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1
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Project Duration: May, 2000 - April, 2003

Abstract:

Listeria monocytogenes is a food borne pathogen, which causes severe disease in humans. *L. monocytogenes* is widely distributed in the environment and is also frequently isolated in food processing environments. Post-processing contamination of heat treated ready-to-eat foods (especially deli meats and sausages) is thought to be the major cause of food borne listeriosis.

Microorganisms that attach and proliferate on solid surfaces are commonly described as biofilms. Biofilm formation has been reported in the food industry and it has been demonstrated that microorganisms growing in biofilms may be highly resistant to common cleaning and sanitizing procedures. Biofilm formation by *L. monocytogenes* may enhance their persistence in food processing environments giving the organism a higher chance to consistently contribute to post processing contamination of heat treated meats.

It is hypothesized that *L. monocytogenes* strains frequently implicated in human disease (epidemic strains; serotype 4b) have a better ability to form biofilms than environmental strains (sporadic strains; serotypes 1/2a, 1/2b, 1/2c) that are less often involved in disease. The research addresses this question and evaluates strain to strain differences of *L. monocytogenes* for biofilm growth and sanitizer resistance.

Analysis of adhesion over 3 hours and biofilm formation over 24 hours for 21 *Listeria monocytogenes* (*L.m.*) strains (encompassing both epidemic and sporadic isolates) clearly showed that epidemic strains adhere in higher numbers than sporadic strains. However, there was no significant difference in cell numbers for 24-hour biofilms.

To explain this difference in adhesion, the cell surface properties of the 21 *L.m.* strains were characterized. Cell surface hydrophobicity, cell surface charge and amount of extracellular carbohydrates produced, are factors that may affect adhesion of microorganisms to solid surfaces. There was significant variation in cell surface hydrophobicity and cell surface charge between strains, but the strain differences did not match the grouping of epidemic and sporadic strains. Extracellular carbohydrates of planktonic cells and of cells after 3h attachment were determined by colorimetric method. There was a significant higher amount of carbohydrates present in cells after 3-hours adhesion than in planktonic cells. Interestingly, adhered epidemic strains contained significantly more carbohydrates than sporadic strains. This difference was not observed for planktonic cells. The higher initial adherent ability of epidemic strains may be caused by higher carbohydrate production during adhesion.

The research compares biofilm formation of *Listeria monocytogenes* strains associated with human disease with that of strains isolated from foods and food processing environments. The knowledge gained will help to address the question whether biofilm formation in food processing environments could enhance the ability of *L. monocytogenes* to colonize the food processing environment, resulting in persistent food contamination and potentially food borne disease. A better understanding of the role of biofilms in the incidence of food borne listeriosis will help the food industry and government agencies to specifically target intervention strategies to the most important areas in the food production chain.

Risk Control/Risk Management Category (RCM)

RCM01 - Project Title: A community trial to determine an effective intervention for the delivery of HACCP to the food service sector

Project Leader: Dr. Tom Abernathy, Central West Planning Information Network - Hamilton, 10 George Street, #301B, Hamilton, ON L8P 1C8 Tel: 905-570-9952 x239 Email: toma@cwhpin.ca

Collaborating Establishment(s): Brant County Health Unit, Haldimand-Norfolk Regional Health Department, Halton Regional Health Department, City of Hamilton Social & Public Health Services Division, Regional Niagara Public Health Department, Waterloo Region Community Health Department, Wellington-Dufferin-Guelph Health Unit

Project Duration: September, 2001 - March, 2003

Abstract:

Food-borne diseases are important contributors to human morbidity and mortality, and result in a significant cost to the health care sector. Recent changes in eating habits and the tendency for people to eat more of their meals outside of the home only increase the risk posed by these illnesses. Consequently, maintaining confidence in the food prepared and consumed in commercial establishments has become an important element in any food safety system. Ontario food premises inspection programs traditionally have focused on diminishing risk and improving compliance with the design criteria and basic sanitation requirements in the Food Premises regulation under the *Health Protection and Promotion Act*. While this approach has been successful in achieving improved maintenance and cleaning of equipment and facilities, it has not allowed for consistent evaluation of food preparation processes in food premises. Despite interest in food safety programs with proven effectiveness, a recent systematic review of the literature revealed that few such examples exist.

The goal of this project is to evaluate the effectiveness and applicability of a food safety program for the food service industry based on HACCP (Hazard Appraisal Critical Control Point) principles. Originally developed for use in the food processing industry to ensure that all food consumed is safe to eat, HACCP is a structured process control system that identifies where hazards might occur in the food production processes and puts into place stringent actions to prevent them from occurring. The intervention to be tested (The Critical Approach) is one developed in collaboration and consultation both with public health inspectors and service managers. It involves an initial visit with operators to explain the principles of HACCP and introduce the program; a consultation to demonstrate how they might apply to their operation; and a follow-up visit to address any issues with implementation or compliance.

The evaluation will take the form of a community trial in which the program to be tested will be introduced to a random sample of 30 restaurants in three communities in Central West Ontario. The control group will consist of 60 restaurants with similar characteristics in other similar communities. A team of independent and 'blinded' auditors will carry out both Pre and Post - Implementation assessments. Results will be shared with public health units in the province, operators of food service premises, the provincial government and the public health community through presentations, reports and published articles.

RCM02 - Project Title: Enhancing the safety and quality of poultry products

Project Leader: Dr. S. Barbut, Department of Food Science, University of Guelph, Guelph ON N1G 2W1
Tel: 519-824-4120 x 3669 Email: sbarbut@uoguelph.ca

Project Duration: May 1999 - April 2003

Abstract:

This project is designed to assist the Ontario poultry industry in improving the quality and safety of fresh and further processed meat products. The project will focus on four areas: 1) cause and potential use of birds condemned for cyanosis; 2) reduction of microbial load in fresh meat and further processed products; 3) sensors to control meat processing parameters to increase production efficiency and quality control; and 4) study the use of different light sources to increase the acceptability of meat products at the retail level.

RCM03 - Project Title: Improving the safety of fermented meat products

Project Leader: Dr. S. Barbut, Department of Food Science, University of Guelph, Guelph, ON N1G 2W1
Tel: 519-824-4120 x 3669 Email: sbarbut@uoguelph.ca

Collaborating Researchers: Dr. M. Griffiths, Department of Food Science, University of Guelph

Project Duration: May, 2000 - April, 2004

Abstract:

Foods of bovine origin continue to be associated with the majority of hemorrhagic colitis derived from *E. coli* O157:H7 food infections. The most recent recall, involving 19 million lb, took place in August 2002 after the pathogen was discovered in meat produced by one of the largest meat companies in the USA (Con Agra). Dry fermented meat products have also been associated with the pathogen, since it is an acid adaptive and acid tolerant microorganism i.e., the bacteria can survive some of the fermentation and drying steps of this un-cooked dry product. Currently, the Canadian Food Inspection Agency is requiring processors producing dry fermented sausages to follow one of five avenues to assure that the pathogen does not survive in the product or pose an unacceptable risk to the consumer.

The project is designed to assist the Ontario meat industry in validating and improving safety of uncooked, fermented meat products. The program will focus on two areas: 1) validation of existing processes employed by Ontario manufacturers; and 2) development of mathematical model(s) to assist in evaluating existing processes and for designing new procedures.

This study will assist processors in validating their processes to meet the government requirements. In addition, the results from various studies and mathematical models will be grouped to assist the industry in reducing the need for conducting so many validation studies. This can benefit the industry since currently a change in any processing parameter (e.g., casing diameter, salt level) requires a new, and fairly expensive validation study.

The overall objective of the work is to assist the Ontario meat industry in producing safer fermented meat products and becoming more competitive.

RCM04 - Project Title: Alternatives to raising pigs without antibiotics

Project Leader: Dr. C. Dewey, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. M.Z. Fan (Department of Animal Science), Dr. D. Hodgins (Department of Veterinary Microbiology & Immunology)

Project Duration: May 2000 - April 2003

Abstract:

The overall purpose of this research is to evaluate alternatives to routine use of in-feed antimicrobials for nursery age pigs. Growth promotion, disease prevention, and disease treatment will each be examined. This study is taking a multi-disciplinary approach to answering some of the questions raised by the possibility of raising nursery pigs without the benefit of in-feed antibiotics. The three principle investigators involved with this study: Drs. Ming Fan, Douglas Hodgins, and Cate Dewey have training in neonatal nutrition, immunology and veterinary epidemiology respectively. This diverse training and experience will provide an opportunity to approach this one very large project from many different aspects.

RCM05 - Project Title: Modelling the survival of enterohemorrhagic *E. Coli* in fermented meats and other meat products

Project Leader: Dr. M. W. Griffiths, Canadian Research Institute for Food Safety, Department of Food Science, University of Guelph, Guelph, ON N1G 2W1 Tel: 519-824-4120 x2269 Email: mgriffit@uoguelph.ca

Collaborating Researcher: Dr. Shai Barbut, Department of Food Sciences, Ontario Agricultural College, University of Guelph

Project Duration: April, 1999 - May, 2001

Abstract

The safety of meat products in general, and fermented sausages in particular, have recently been questioned due to several outbreaks of enterohemorrhagic *E. coli* infections resulting in several deaths of young children and seniors. The Canadian industry has not been immune to this problem. There have been at least two documented reports linking fermented sausages produced in Ontario to cases of human illness. These meat-related outbreaks have caused the U.S. government to revise the regulations governing the meat industry, and to ensure continued trade in meat products with the U.S., Canadian processors must comply with these new policies. However, the measures required for compliance are expensive and may be prohibitive for small companies.

The objectives of this research were to 1) develop a model which describes the survival of *E. coli* O157:H7 in meat during the manufacture of fermented sausage; and 2) gain information about the interaction of the lactic acid bacteria present in the starter culture and *E. coli* O157:H7 by using different reporter genes to visualize the different bacteria.

Models were developed to describe the log reduction of *E. coli* O157:H7 in uncooked, semi-dry, fermented sausages. The models focused on different variables to determine which parameters would best describe the response of *E. coli* O157:H7 in these products. The variables examined included time, temperature and product formulation. Fermentation alone produced a mean log reduction in *E. coli* O157:H7 count ranging from 0.30 to 1.33 whereas the mean log reduction in *E. coli* O157:H7 count obtained during the drying stage ranged from 1.37 to 2.70. Overall, the lowest mean log reduction of *E. coli* O157:H7 was 2.05 whereas the highest was 3.36.

Three experiments were conducted in which sausages were made with casing diameters of 55 mm and 90 mm. The rate of pH change was similar regardless of diameter, whereas the water activity decreased about twice as fast in the smallest diameter sausages. Further work is needed to elucidate the relation between casing size and survival of *E. coli* O157:H7.

Work using the bioluminescent strain has indicated that *E. coli* O157:H7 mainly survives in the adipose and connective tissue in the sausage during the drying process. To determine whether microcolonies (nests) of lactic acid bacteria are present in regions that do support the survival of *E. coli* O157:H7, microscopic imaging of gram-stained thin slices of sausages were carried out. These experiments confirmed that lactic acid bacteria are present mainly in the muscle tissue and are not associated with fat.

A part of this work was published and is referenced below:

Pond, T.J., Wood, D.S., Mumin, I.M., Barbut, S. and Griffiths, M.W. 2001 Modeling the survival of *Escherichia coli* O157:H7 in uncooked, semi-dry, fermented sausage. *Journal of Food Protection* 64: 759-766.

RCM06 - Project Title: Avian mucosal lectins and resistance to *Salmonella* and *E. coli*

Project Leader: Dr. M.A. Hayes, Department of Pathobiology, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May 2000 - April 2003

Abstract:

This study will explore some opportunities for control of *Salmonella* and *E. coli* problems in poultry by novel dietary exclusion strategies. Neonatal mammals establish enteric flora while consuming milk containing many oligosaccharides that can competitively exclude particular bacterial polysaccharide or fimbrial lectin surface phenotypes. Chickens can to some extent exclude *Salmonella* if they pre-acquire normal alimentary flora (i.e. competitive exclusion) or are administered mannans that mimic bacterial fimbrial adhesion targets in the alimentary mucosa. This laboratory has discovered two novel plasma and secreted protein lectins that bind acetylated surface polysaccharides of *E. coli* pathogenic to poultry. Various unrelated lectins for other gram negative bacterial pathogens were found similarly in mammals and fish. These proteins and polysaccharide targets enable the discovery of competitors, such as N-acetylated-saccharides. A similar approach will be used to first identify and then use avian mucosal lectins for *Salmonella enteritidis* and *S. typhimurium* surface polysaccharides as assays for competitors. It is expected that mono- or oligosaccharides or related chemicals that compete in ways that are suited as dietary exclusion products will be identified. Reciprocally, if required, a competitive binding assays for bacterial fimbrial lectin-adhesions will be developed and their avian mucosal polysaccharide targets to identify substances that can interfere with this mechanism of mucosal colonization. These screening strategies are expected to lead to the discovery of new dietary constituents/additives that interfere with host and microbial polysaccharide dependent adhesion colonization relationships that underlie host and environmental contamination by problematic *E. coli* and *Salmonella*. This work also has the potential to identify possible avian host resistance phenotypes amenable to genetic selection.

RCM07 - Project Title: A systems approach to the evaluation and extension of ready-to-use packaged vegetables

Project Leader: Dr. Y. Kakuda, Department of Food Science, University of Guelph, Guelph, ON N1G 2W1
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Collaborating Researchers: Dr. Ian Britt, Department of Food Science, University of Guelph, Dr. Robin McKellar, Agriculture and Agri-Food Canada, Dr. Joseph Odumera, Laboratory Services Division, University of Guelph

Project Duration: May, 2000 - April, 2003

Abstract:

Fresh-cut produce for retail, foodservice and institutional markets is rapidly growing as a food packaging application in Ontario. The microbiological safety of these foods is of concern, because of the potential for temperature abuse, especially during distribution and retail/consumer handling. Minimal processing of fruits and vegetables covers a wide range of processing and packaging technologies to preserve food as they move from agricultural production to the consumer. However, all fruits and vegetables are living tissues that continue to respire after harvest. Associated with respiration, the primary spoilage mechanisms that influence shelf life are metabolism of the plant tissue and the growth of microorganisms. These are also associated with enzymatic browning, pink rib, pigment loss, vitamin degradation, chemical changes due to packaging, as well as flavor and aroma changes. The rate of respiration is strongly influenced by temperature and O_2 / CO_2 concentrations within the package. The challenge for extended shelf life is to minimize respiration without creating anaerobic conditions within the package.

The objective of this study is to assess the browning and microbial condition of cut lettuce to determine the impact of using ceramic knives or ultrasonic cutters on shelf life as compared with conventional practice which is to use stainless steel knives. The study focuses on iceberg lettuce packaged in commercially available polymer films but the techniques developed may be applied to other products.

The presence of cellular fluids released from lettuce tissue during harvest and processing may provide sufficient nutrients to support the growth of bacteria, yeast and molds. While relatively few types of pathogenic microorganisms are considered as possible health hazards for fresh-cut vegetables, *Listeria monocytogenes*, *Aeromonas hydrophila* and *Pseudomonas* spp. are psychrotrophic organisms which do occur on fresh vegetables. Also, shelf life is strongly influenced by enzymatic browning that is commonly observed on freshly cut tissue surfaces resulting from enzymatic oxidation of phenolic compounds released as cells are disrupted.

For each storage study, modified atmosphere packaging is used to establish an initial gas composition of 5% O_2 , 5% CO_2 and 90% N_2 to permit rapid attainment of equilibrium conditions in the packages. Storage temperatures of 4 and 10°C, represent optimum and 'abusive' conditions, respectively. During a study, samples are tested over time for total plate counts, psychrotrophic bacteria, coliforms, and yeasts and molds. Oxygen and carbon dioxide concentrations in the headspace are also measured through each storage study to determine if aerobic conditions are maintained during storage and if the processing technique influences respiration rates. Visual quality of the product is assessed by sensory evaluation based on hedonic scoring systems used in earlier studies in our laboratory and through the use of an image analysis system developed by Agriculture and Agri-Food Canada to quantify the degree of browning. Micrographs of the cut surfaces are also being acquired using digital light and scanning electron microscopy to visually assess the efficacy of each cutting system with respect to minimizing cell damage.

RCM08 - Project Title: Enhancing the microbial food safety of pork

Project Leader: Dr. S. McEwen, Department of Population Medicine, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2002 - April, 2003

Abstract:

Food safety is an important issue for the pork industry because it affects public health, consumer confidence and trade. Microbial hazards in food are the most serious food safety problems and must be reduced by efforts at all levels of the food production, processing, distribution and food service system. At the farm level, producers should be developing quality assurance programs to reduce fecal carriage and shedding of enteric organisms of food safety concern. These include drug-resistant bacteria, *Salmonella* and others. Development of useful programs of this type will require new knowledge on the epidemiology of these agents in pigs to identify the antimicrobial usage practices and other management factors that increase risk so that practical risk reduction steps can be taken on-farm. The proposed research is intended to provide such knowledge and will focus on antimicrobial resistant bacteria and *Salmonella*, important foodborne contaminants of pork.

RCM09 - Project Title: Process development for lower-fat and better-quality fried foods using edible coatings and for pan-fried meat patties for better safety

Project Leader: Dr. G. S. Mittal, School of Engineering, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2000 - April, 2004

Abstract:

This 4-year project is directed towards process development through modelling of deep-fat- and pan- frying to improve quality and safety of reduced and regular fat products. Specific objectives are: (a) To determine food quality changes, crust formation, shrinkage during deep-fat frying, and developing mathematical models; (b) To model and validate deep-fat frying of edible-film coated foods, optimize process conditions for low-fat foods, and develop artificial neural network (ANN) for online process control and process optimization; (c) To develop and validate models to predict heat, moisture and fat transfer, quality kinetics, and microbial lethality in beef chicken patties during single-sided pan-frying with variable heating plate temperature, heating sequence, frequency of turn-over, and changes in patty composition; and (d) To develop process conditions for different frequency of turn-over and fat content for better safety and quality of pan-fried foods.

To reduce fat uptake in fried foods, a novel concept of composite coating was developed. More than 90% fat uptake can be reduced in fried foods when applying these coatings to products before frying. Out of many materials evaluated, four combinations were found to be suitable: soy-protein isolate + whey protein (99.8% $\pm$ 9.0% fat reduction), whey protein + sodium caseinate (95.6% $\pm$ 4.6%), whey protein + methyl cellulose (93.6% $\pm$ 5.9%) and soy-protein isolate + whey protein (91.4% $\pm$ 6.6%). These coatings provided low-fat fried-foods with negligible change in texture and taste. Appropriate crust was also formed which is typical for fried foods.

ANN-based models were developed to predict heat and mass transfer during deep-fat frying of foods coated with edible films. The models were in good agreement with experimental results. These models can also be used in industrial fryer controls for accurate predictions of temperature, and fat and moisture contents of foods. All of these factors are important in microbial lethality.

Frying is an important and large food operation in Ontario. Modern frying systems are becoming complex due to industry's expanding need for higher production, short cooking time, consistency and better quality, higher yield, energy efficiency and versatility. Process models will provide a better understanding of the process, and assist with better design of equipment and processes, and will promote more efficient use of existing equipment. Optimization of the process parameters will result in better process control, product quality and lower fat. As the market for fully cooked products continues to expand, modelling and simulation that predict product temperature, cooking yield, and pathogen lethality during cooking will be valuable tools for designing effective and safe thermal processes. ANN is a useful tool for real-time prediction of data and for online process control. With the increasing concern on safety and quality of beef patties (hamburgers), the importance of the prediction of heat and fat transfer, microbial lethality, and optimization of the pan-frying process is evident. Single-sided pan-frying or roasting may not provide slowest heating point at patty geometric centre due to different temperatures on both patty sides. Different patty fat content and the frequency of turn-over of patties make this problem more difficult.

RCM10 - Project Title: "Reduced Risk" herbicides

Project Leader: Dr John O'Sullivan, Department of Plant Agriculture, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May 2000-April 2006.

Abstract:

The registration of new weed control products is of great significance to vegetable growers. It reduces the need for labour for hand-hoeing and weeding, reduces the cost of production to the grower and improves competitiveness of this industry by providing more cost effective weed control, resulting in economic benefit to the growers. It provides vegetable growers access to newer, potentially safer and more effective herbicides and minimizes the impact of herbicide application on the environment. It provides vegetable growers other weed control options and encourages a more sustainable production system by utilizing environmentally friendly herbicides. The objective of the proposed research is to evaluate new, low-use rate, "reduced-risk", environmentally-compatible herbicides for weed control efficacy and crop safety in vegetables to develop data to facilitate registration. Registration of these products for use in Ontario will ensure that Ontario vegetable growers do not have an unfair advantage with growers elsewhere who compete with them. The implementation of the new Food Quality Protection Act (FQPA) has potential to result in the loss

of a large number of minor uses. The problem of insufficient pest management options for minor crops will be compounded as a result of FQPA risk considerations. FQPA aims to promote "reduced risk" pest management systems. This will focus on bringing "reduced risk" safer products to the market. This project will also help to make available "reduced risk" and safer weed control products for growers of low acreage crops. The availability of this new weed control technology will help growers of low acreage crops become more competitive. Registration of "reduced risk", safer herbicides will meet Ontario Government requirements for impact on the environment. The availability of "reduced risk", safer herbicides that are low-use rate, environmentally friendly and of low toxicity to humans will contribute to overall pesticide reduction.

The study objectives include:

1. Evaluate new "reduced risk" herbicides for weed control in low acreage crops.
2. Develop efficacy, crop safety and residue data, especially for new, low-rate, environmentally-compatible herbicides to support product performance data for registration and labelling.
3. Make available "reduced risk" and "safer" weed control products for growers of "minor" crops by obtaining registration of "minor" use products under URMULE. These weed control products offer "low-risk" alternatives to benefit vegetable growers.

RCM11 - Project Title: Implementation of food safety quality assurance schemes and an assessment of appropriate labelling for Ontario fruits and vegetables

Project Leader: Dr. Douglas Powell, Department of Plant Science, University of Guelph, Guelph, ON N1G 2W1
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Project Duration: May, 2002 - April, 2003

Abstract:

Increasing consumption of fruits and vegetables coupled with an increasing awareness of the potential for such commodities to cause foodborne illness has led to local and national on-farm food safety initiatives in both Canada and the U.S. This project will continue to assist Ontario producer groups with the implementation of effective and meaningful on-farm food safety programs, to establish a certification program for growers, and to evaluate various labeling schemes in a commercial setting to help illustrate Ontario producer commitment to safe, high-quality food and ultimately enhance consumer confidence.

Ontario producer attitudes and perceptions will be evaluated using standard risk perception surveys and factor analysis. HACCP or on-farm food safety plans will be updated in accordance with international guidelines and in a manner consistent with evolving recommendations from American and Canadian federal authorities. Risk messages targeted at both producers and employees will be iteratively developed and tested using focus groups of producers and employees.

Consumer research to identify meaningful labeling schemes will employ triangulation methods using both qualitative and quantitative research methods to gain fuller understanding of consumer perception, attitudes and behaviors as well as how certain communication strategies affect these.

A consumer survey will be conducted as an intercept survey at grocery checkout and farmers market checkouts. This will provide quantitative data on consumer attitudes and perceptions of various certification claims and food safety. It will also attempt to identify whether consumers read certification labels posters and what influenced purchase behavior. This will be compared to attitudinal surveys to determine any differences between what consumers say and their actual purchase behavior.

A series of focus groups will be conducted to gain a richer understanding of consumer attitudes and perceptions. They will also be used to evaluate different labeling and communication schemes that exist to test the prototype label.

RCM12 - Project Title: Development of a food safety risk analysis model for issues management and risk communication

Project Leader: Dr Doug Powell, Department of Plant Agriculture, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May 2000-April 2006.

Abstract:

Consumer concerns about food safety, including microbial hazards, biotechnology, environmental impacts, medical implications and general food production practices, are increasing. The Food Safety Network at the University of Guelph provides research, insight

and commentary on all aspects of the farm-to-fork food chain. Using electronic networks, databases, field research, and consumer outreach, the Food Safety Network works closely with the Canadian Research Institute for Food Safety, as well as other collaborators, to develop and implement scientific and publicly credible policies and programs to enhance the safety of the food supply. LISTSERV, (electronic communications tools to assist in risk analysis activities) rapidly identifies issues for risk management and communication activities, promote awareness of public concerns in scientific and regulatory circles, and exchange timely and current information for direction of research, diagnostic or investigative activities. This information will be used by the Food Safety Network to create risk communication items such as fact sheets, opinion articles and press releases. The proposed research will examine the effectiveness of the Food Safety Network as a risk analysis and risk communication tool to enhance consumer confidence in the safety of Ontario's food. This research will provide fundamental, long-term insights into how best to meet changing consumer expectations with the development of a flexible and rigorous food safety risk communication model.

This study objectives include:

1. Publish LISTSERV on a daily basis as a source for food related risk analysis information to feed into risk communication activities.
2. Publish a weekly risk communication vehicle e.g. opinion article, fact sheet, demonstration video, etc., on a specific aspect of food safety risk analysis.
3. Continually update and enhance the Food Safety Network website, including the searchable database.
4. Conduct two focus groups and/or personal interviews per year with representative consumers and professionals to assess food safety risk communication vehicles and messages.
5. Develop retrospective case studies of food safety issues management, such as labelling of genetically engineered foods, and outbreaks of *E. coli* 0157:H7, involving Food Safety Network material.
6. Create a model of Food Safety Network risk communication activities and test this model with at least three different agri-food organisations.

RCM13 - Project Title: Implementation and evaluation of on-farm food safety programs for the Ontario fruit and vegetable sector

Project Leader: Dr. Doug Powell, Department of Plant Agriculture, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May 1998 - April 2002.

Abstract:

An integrated risk management and communication protocol, on a commodity-specific basis, was developed and implemented to ensure safe fruit and vegetable production and optimal public confidence. An on-farm food safety program was developed, implemented and analyzed for the Ontario Greenhouse Vegetable Growers in Ontario, over a two-and-a-half-year period. This HACCP-based system was designed to reduce the potential for microbial contamination along the entire production and distribution process. The On-Farm Food Safety Program was designed to educate operators on how to identify and control any microbiological threats. Growers were provided with a program manual that gave them a better understanding of food safety. Areas focussed on were water supply, equipment, worker hygiene, storage and transport of products, sanitation and pest control. Water samples were tested for total coliforms, *E. coli* and *Salmonella*. Water treatment methods, worker hygiene and general sanitation was found to be adequate in the Leamington area. This suggests that the on farm food safety program is working. Research is ongoing on appropriate and adequate water treatment methods as well as investigating proper protocols for recalls. The livelihood of the greenhouse growers depends on the safety of their consumers. This program will eventually result in grower certification where consumers are guaranteed a safe high quality product. Through the use of microbiological testing, on-site visits and producer surveys it was determined that the program has increased grower knowledge, understanding and awareness of microbial risks associated with fresh produce and caused improvements in practices used within the greenhouse and packing sheds. This project is the standard for produce-related, on-farm food safety programs in North America. The research has identified the significant awareness and communications barriers that must be overcome, the need for practical implementation, and the importance of producer-led solutions to successfully implement an on-farm program. This work is now being expanded to OFVGA, Ontario Processing Vegetable Growers (OPVG) and others within the farm-to-fork food safety system. To remain competitive in national and international markets, all levels of the Ontario fruit and vegetable industry must strive to minimize microbial and chemical contamination. Fresh fruits and vegetables are increasingly recognized as vectors for foodborne illness. Prevention is especially important during production where many of these contaminants enter the food chain, especially as evidence mounts that washing is largely ineffective at removing many pathogens.

RCM14 - Project Title: Use of prebiotics and probiotics as replacements for antibiotics in pig starter feeds

Project Leader: Dr. Gregor Reid, Lawson Health Research Institute, 268 Grosvenor Street, London, ON N6A 4V2
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Collaborating Researchers: Dr. Robert Friendship, Department of Population Medicine, University of Guelph and Dr. Cornelius de Lange, Department of Animal and Poultry Science, University of Guelph

Project Duration: July, 2001 - June, 2002

Abstract:

There is growing evidence to show that probiotics and prebiotics can play a role in developing a healthy fecal bacterial flora in pigs, thereby helping to resist infection, reduce pathogen shedding, lower the risk of development of antibiotic resistance, while still maintaining good growth performance.

This study is exploring the use of freeze dried and liquid feed forms of experimental and commercial probiotics for prevention of *E. coli* diarrhea, and as a substitute for antibiotics to obtain good weight gain in weanling pigs. Several hundred post-weaning animals have been studied, some challenged with *E. coli* K-12 and others allowed to become infected with naturally occurring *E. coli* known to afflict most herds.

Denaturing gradient gel electrophoresis, so far, has not detected any major changes in the types of bacteria inhabiting the gut before, during or after pathogen challenge. The next phase will determine whether proportionate viable counts alter with antibiotic or probiotic use. Studies indicate that lactobacilli can reduce inflammation and enhance protection from infection including reducing pathogen translocation across the intestinal wall, thus, we will examine mucosal epithelia for inflammatory changes. To date, antibiotic laced feed have not provided any detectable advantage to the health and weight of the pigs. A commercial probiotic has not shown promise in pilot studies but further work is underway. Freeze dried capsules proved not to be a good method of delivery. Dried probiotics are commonly used by industry, but the viable count tends to drop rapidly unless the probiotic organisms are protected. These issues have led us to develop a liquid feed method that should provide a practical system for producers to feed their animals on site. This feed comprises a strain newly isolated from a healthy animal, plus proven human and animal probiotic strains *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14.

This research has the potential to generate intellectual property which could stimulate manufacturing and global sales of probiotics for pigs and other livestock. Given that Europeans have successfully stopped the use of antibiotics for weight gain, the concern over emergence of multi-drug resistant 'superbugs', and consumer dissatisfaction with drugs being used in their food chain, probiotics could provide an excellent alternative to improve the health of livestock. The strength of the livestock industry in Ontario along with the presence of the Canadian Research and Development Centre for Probiotics provides a wonderful opportunity for the sector to develop new global products and strategies. Even a conservative 10-20% improvement would be significant as an estimated \$200-300 million of revenue is lost in Canada each year due to premature deaths in pigs caused by gut infections.

RCM15 - Project Title: Removal efficiency of representative bacterial and protozoan pathogens from cattle and pig farm wastewater using wetland technologies

Project Leaders: Dr. Robin Slawson and Dr. Barry G. Warner, University of Waterloo, 200 University Avenue West, Waterloo, On N2L 3G1 Tel: 519-888-4567 x 6033 Fax: 519-725-9971 Email: lynnico@uwaterloo.ca

Collaborating Researchers: Dr. R. Aravena

Project Duration: January, 2002 - December, 2004

Abstract:

Wetland technology is emerging as a cost-effective and environmentally friendly option for treating wastewater and improving water quality. Organic-rich agricultural wastewater has been shown to be well suited to treatment by wetland technology. The ability of wetland technologies to remove pathogens from agricultural wastewater is less well known, although research on the removal of pathogens from domestic wastewater by wetlands has been shown to be effective.

This project aims to monitor the wetland removal efficiency for pathogenic enteric bacteria (*Escherichia coli*, *Salmonella*, *Campylobacter*), and protozoa (*Cryptosporidium*) in representative subsurface flow treatment wetlands systems on a cattle and on a pig farm in southwestern Ontario. The former is situated on the southern part of the Region of Waterloo, a region with a high concentration of dairy farms, and the other is situated in Huron County, a region with a high concentration of pig farms. Each of these farms has or will have a subsurface flow treatment wetland system operating in them to deal with point source wastewater from the barnyards.

The primary objective of this project is to characterize the spacial distribution and year round temporal variability of pathogenic enteric bacteria (*Escherichia coli*, *Salmonella*, *Campylobacter*) and protozoa (*Cryptosporidium*) on soil particle, in the soil water and on the plant roots (rhizosphere). Wastewater from a pig farm versus a cattle farm will be compared for efficiency of the wetland to remove these pathogens. A network of sampling points, both shallow and deep, will be setup in each of the wetland cells operating are each of the farm sites. Monthly sampling will be undertaken. This two-level factorial design will aid in assessing the significance of plant root material in microbial establishment and pathogen removal, as well as the influence of seasonal temperature on the population development.

RCM16 - Project Title: Vaccination of cattle to reduce fecal shedding, environmental and water contamination with *E. coli* O157:H7

Project Leader: Mr. Martin Warmelink, President Food Safety, Bioniche Life Sciences Inc., P.O. Box 1570, Belleville, ON K8N 5J2 Tel: (613) 966-8058 Email:

Collaborators: Ontario Cattlemen's Association, University of Guelph

Project Duration: January, 2002 - January, 2003

Abstract:

Escherichia coli O157:H7 is a major cause of foodborne illnesses in Ontario. It is perhaps our most important food safety problem, because it can cause very severe, sometimes fatal infections, children are at greatest risk, and it is responsible for many financial losses from food recalls and diminished consumer confidence. A vaccine has been developed in western Canada with the intent of administering to cattle to reduce the prevalence of *E. coli* O157:H7. A trial is now underway in large beef lots in western Canada. The applicants are proposing to determine the prevalence and level of fecal shedding, water and farm environmental contamination with *E. coli* O157:H7 on Ontario cow-calf operations. The applicants will examine the effects of whole-herd vaccination on the prevalence and level of fecal shedding, water and farm environmental contamination with *E. coli* O157:H7 on Ontario cow-calf operations.

The results are to be published in peer-reviewed scientific publications, presented at industry conferences and used to gain registration for this vaccine in Canada. Following this, a facility would be established in Ontario for the manufacture and distribution of the vaccine world-wide, creating jobs and export opportunities within the province.

RCM17 - Project Title: Development of bovine specific probiotics for the prevention and treatment of *Escherichia coli* diarrhea in calves

Project Leader: Dr. J. S. Weese, Department of Clinical Studies, University of Guelph, Guelph ON N1G 2W1
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Project Duration: May, 2002 - April, 2003

Abstract:

Probiotic therapy is receiving increasing attention in veterinary medicine as a safe, non-antibiotic and potentially efficacious method for treatment or prevention of a number of diseases. This project will identify bovine-specific probiotics for the elimination of *E. coli* H7:O157 carriage and shedding in dairy cows. This study will isolate and identify 100 lactic acid bacteria from normal cattle. Bovine-origin bacteria will be used as it is presumed that they will be better adapted to colonize the intestinal tract compared to bacteria originating in other species. Isolates will be tested for the ability to survive acid and bile environments, as would be encountered in the abomasum and small intestine. Isolates will also be tested for aerotolerance, to ensure that they can survive processing and transportation from the laboratory to the animal. The ability to produce an antimicrobial factor against strains of *E. coli* O157 will also be evaluated. Isolates will then be freeze dried and administered orally to adult cows to determine whether they are able to survive intestinal transit in vivo at different doses. Rectal mucosal biopsies will be used to assist in determining whether isolates actually colonize the intestinal tract. Isolates possessing all these beneficial properties will be used in future studies evaluating their ability to eliminate *E. coli* O157 shedding in adult cows. Based on a study looking at anti-*Salmonella* and anti-*E. coli* probiotics in horses that is ongoing in this laboratory, it is anticipated that 2-4 isolates with all the above described properties will be identified.

RCM18 - Project title: Application of ozone for improving food quality and microbial safety

Project Leader: Dr. H. Zhou, School of Engineering, University of Guelph, Guelph ON N1G 2W1
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Collaborating Researchers: Dr. T. Zhou and Dr. R. McKellar, Agriculture and Agri-Food Canada

Project Duration: May, 2002 - April, 2004

Abstract:

Risks from microbial contamination and the potential to form toxic by-products during disinfection with chlorinated compounds are concerns in the food industry. Ozone, being widely used as an alternative disinfectant to chlorine in water treatment, has been demonstrated to be a promising technology which could be used in food industry to ensure microbial safety and quality of foods. It has powerful disinfection ability, high reactivity and spontaneously decomposes to non-toxic oxygen. To properly apply this technology in practice, research is needed to quantify the effectiveness of ozonation for different food products under different scenarios and provide the necessary design protocols. Thus, the research objectives of this project include:

- 1) to examine the effectiveness of ozone technology for processing different vegetables and fruits by measuring the inactivation efficiency of selected common and emerging microorganisms,
- 2) to examine the effects of various process conditions including, but not limited to, ozone doses, contact time, ozone application modes, temperature, pH and the presence of interfering materials,
- 3) to develop and quantify the inactivation kinetics and the mass transfer rate of ozone when used in food industry,
- 4) to assess food quality other than microbial contamination after continuous ozone exposure, and
- 5) to provide Ontario food producers with detailed application protocols and specification to properly use ozone as a viable disinfectant in food treatment, processing and preservation.

The results of this research may provide Ontario food processors with a new preservation technology based on the use of ozone as a disinfectant. As well, the effectiveness, limitations and operating protocols of ozone application in food applications will be summarized. The technology also has the potential to reduce wastewater generation via processing water reuse.

Section Three: Quick Searches

Subject	Abstracts	Subject	Abstracts
Antibiotics	DM- 12; DM-21; DM-22	<i>Giardia</i>	DM-05
Antimicrobial resistance	RA-14;RA-15; RA-16 RCM-08	<i>Listeria</i>	DM-04; DM-09; DM-17 RA-19 RCM-07
Beef	DM-04; DM-18 RA-10; RA-13; RA-14; RA-15 RCM-16	Metals	RA-08
<i>Campylobacter</i>	DM-04; DM-06; DM-11; DM-15 RA-02; RA-16 RCM-15	<i>Mycobacterium paratuberculosis</i>	DM-01; DM-13 RA-11
Chemical contaminants	RA-04 RCM-10	Poultry	DM-11; DM-17; DM-18; DM-15; DM-20 RA-02; RA-16 RCM-02; RCM-03; RCM-06
Coliforms	DM-19 RA-17 RCM-13	Processed foods	DM-17 RA-19 RCM-01; RCM-02; RCM-03; RCM-05;RCM-09
<i>Cryptosporidium</i>	DM-05; DM-09; DM-10 RA-18 RCM-15	<i>Salmonella</i>	DM-04; DM-05; DM-06; DM-07; DM-08; DM-09; DM-18 RA-07; RA-12; RA-13; RA-14 RCM-06; RCM-08; RCM-13; RCM-15
<i>Cyclospora</i>	DM-05;DM-16	Swine	DM- 12; DM-21 RA-07; RA-09 RCM-04; RCM-08; RCM-14
Dairy	DM-01; DM-13 RA-11; RA-12; RA-18 RCM-17	<i>Toxoplasma gondii</i>	RA-07
<i>E. coli</i>	DM-02; DM- 03; DM-04; DM-05;DM-06; DM-08; DM-14; DM-09; DM-23 RA-06; RA-07; RA-09; RA-10; RA-12; RA-13; RA-14 RCM-03;RCM-05; RCM-6; RCM-13; RCM-14; RCM-15; RCM-16; RCM-17	Viruses	DM- 03; DM-04 RA-07
Fruits and vegetables	DM-16; DM-17 RA-04; RA-08 RCM-07; RCM-10; RCM-13;RCM-11; RCM-18	Water	DM-02; DM- 05; DM-14; DM-19; DM-10; DM-23 RA-04;RA-06; RA-17 RCM-15; RCM-16
		<i>Yersinia</i>	RA-07